Environmental enrichment for healthy and Alzheimer's disease-associated pathological ageing

Kimberley Stuart

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Faculty of Health

University of Tasmania

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Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Co-authorship

The following people contributed to the publication of work undertaken as part of this thesis.

Author details

1. Miss Kimberley Stuart, Wicking Dementia Research and Education Centre, Faculty of Health, University of Tasmania

2. Dr Anna E. King, Wicking Dementia Research and Education Centre, Faculty of Health, University of Tasmania

3. Dr Carmen M. Fernandez-Martos, Wicking Dementia Research and Education Centre, Faculty of Health, University of Tasmania

4. Mr Justin Dittmann, Wicking Dementia Research and Education Centre, Faculty of Health, University of Tasmania

5. Assoc. Prof Mathew J. Summers, School of Social Sciences, University of the Sunshine Coast

6. Prof James C. Vickers, Wicking Dementia Research and Education Centre, Faculty of Health, University of Tasmania

Author roles:

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Candidate was primary author (65%), and contributed to the design of the study, wrote initial drafts of the manuscript, undertook the laboratory studies, and conducted the data analysis; Author 2 contributed to the conceptualization and design of the project, and writing of the manuscript (10%); Author 3 assisted with laboratory studies (5%); Author 4 contributed to design and construction of materials used (5%); Author 5 contributed to the study design and data analysis (5%); Author 6 formulated and contributed to the design of the project, and the writing of the manuscript (10%).

Signed:

Date: 21st December 2016

Prof James Vickers

Primary Supervisor

Co-Director

Wicking Dementia Research and Education Centre

Faculty of Health, University of Tasmania

Papers and Posters Presented at Conferences

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Abstract

Interventions to reduce the burden of dementia-causing diseases are urgently needed given global ageing, and the current lack of effective therapeutic approaches. Alzheimer's disease (AD) is a neurodegenerative disease, and the most common cause of dementia among older people, for which there is no known cure, or effective treatment. Engagement in complex cognitive activity can promote an array of neuroplastic mechanisms associated with cognitive protection. Epidemiological evidence suggests a high level of cognitive engagement, particularly in early-life, to be associated with a reduced risk of developing dementia in later-life, including that caused by AD. However, an intervention aimed at preventing or delaying dementia is more likely to be taken up in later-life. Furthermore, limitations inherent in epidemiological investigations means that the mechanisms underlying this putative relationship are not well understood, and the array of extraneous factors that are encountered over a person's life-span cannot be controlled for.

In the current study, an environmental enrichment (EE) paradigm was used, in order to experimentally recapitulate the promotion of neural plasticity from experience. The overarching aim of this study was to investigate the effect of EE in healthy ageing, and on AD-associated pathological ageing after the onset of disease-induced pathology, following a non-stimulating early-life. Healthy wildtype (Wt) and transgenic AD ($APP_{SWE}PS1_{dE9}$) mice were raised in a non-stimulating environment (standard housing; SH) and then randomly assigned back into SH or into EE starting at mid-life (6 months) or later-life (12 months) for a 6-month period. Further, it was aimed to: assess the effect of mid-life EE on hallmark AD neuropathological and structural brain alterations, and how this related to cognitive features, and how this differed to healthy ageing; to investigate the effect of a complex and novel EE paradigm (EE+) on Aβ

neuropathology; and to examine whether the brains' immune cells, microglia, have a role in the link between EE and cognitive protection in a late-life intervention.

The key conclusions drawn from these investigations were that mid-life and later-life EE were not effective interventions in reducing Aβ pathological burden in the presence of existing pathology. However, the mid-life EE paradigm was associated with compensatory processes in AD mice, demonstrated by a benefit to short-term memory, an increase in the neurotrophin BDNF in the hippocampus, and an increase in synaptic density in the CA1 subregion of the hippocampus. The EE+ paradigm was introduced to assess whether more complex activity would be associated with reduction to Aβ pathological burden. Conversely, following the complex and novel paradigm in mid-life, AD mice developed exacerbated Aβ neuropathological burden, and demonstrated an increased vulnerability to stress. APP/PS1 mice were found to have an increase in the area occupied by microglia in the hippocampus and neocortex relative to healthy ageing Wt mice, however, late-life EE altered this effect, resulting in no genotype differences. Overall, this investigation demonstrated EE paradigms introduced through mid to later-life for both non-pathological and pathological ageing, are associated with some level of cognitive enhancement. The findings of this thesis suggest the protective effect of EE on cognitive function in a transgenic model of AD pathology, relies on an enhancement of synaptic connectivity and factors that promote neural plasticity, as cognitive protection was observed with no evidence of an attenuation of existing Aβ neuropathology.

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Chapter 1

1. Introduction

Neural plasticity describes the capacity of neurons and neural circuits to undergo structural and functional alterations in response to experience. Over the late 19th century, the view of the adult brain being a fixed entity was replaced by the now prevailing view of the adult nervous system being highly adaptive, and capable of exhibiting plasticity well past the sensitive "critical periods" during early development (Fuchs & Flügge, 2014). However, there is evidence that we have long been aware of the effect of experience from the environment on the neural system, with the idea that a lack of stimulation would cause neural processes to withdraw from each other posited as early as the 19th century (Bain, 1872; Cajal, 1894; as cited in Rosenzweig, 1996). In a time of population ageing, with a concomitant higher vulnerability to neurodegenerative disease and cognitive deterioration, it is critical to understand how to harness and promote neural communication and enhance the plastic capacity of the ageing nervous system.

The global ageing of populations is driving a substantial increase in the presence of dementia. Dementia describes a syndrome in which there is a progressive loss of memory, cognition, changes to behaviour, and an impaired ability to perform everyday activities (Sosa-Ortiz, Acosta-Castillo, & Prince, 2012). Although once thought to be an inevitable part of ageing, it is possible to experience ageing without dementia. However, with age, the nervous system becomes increasingly vulnerable to conditions that cause dementia (Hofer, Berg, & Era, 2003). Ageing can occur non-pathologically, accompanied by "normal" ageing-related decline, as opposed to pathological-induced decline observed in diseases such as Alzheimer's disease (AD; Hedden & Gabrieli, 2004). Normal cognitive ageing is heterogeneous, however, there is a typical pattern of decline to processing speed, episodic and working memory, while short-term and

autobiographical memory, semantic knowledge, and emotional processing typically remain stable (Hedden & Gabrieli, 2004; Salthouse, 2011). Observed in those with dementia, is earlier memory impairment, followed by incipient global cognitive decline (Weintraub, Wicklund, & Salmon, 2012). While more constrained in later life, the brain has capacity to alter its structure and function over the entire lifespan (Jellinger & Attems, 2013). Why differing trajectories to normal cognitive ageing, or a decline into pathological cognitive ageing are experienced is not well understood, however, a complex interaction of biology and the environment is likely.

1.1 Alzheimer's disease

Alzheimer's disease (AD), clinically described as an ultimate progression of global cognitive and behavioural impairment, the disease has no current effective treatment or cure (Prince et al., 2015). AD is the most common cause of dementia, and is also one of the mostly widely studied forms of neurodegenerative disease (Hardy, 1997; Vickers et al., 2000). It was over a century ago that Alois Alzheimer first detailed the clinical expression of the disease in his patient Auguste D, and further described the presence of abnormal structures within the brain tissue. Despite concerted research efforts over the last century, there is no clear understanding of the fundamental underlying pathological processes of the disease and how this is related to the clinical outcome of dementia.

The pathological interpretation of AD focuses on the presence of the abnormal structures as detailed by Alzheimer, described as the pathological hallmarks of the disease. Held in the extracellular space of an AD brain, are fine filaments composed of β-amyloid (Aβ) that accumulate to form plaques. Observed in cell bodies are aggregates of abnormally hyperphosphorylated and aberrantly misfolded tau that comprise

neurofibrillary tangles (Vickers et al., 2000; Woodhouse, Vickers, Adlard, & Dickson, 2009). While Aβ plaques and neurofibrillary tangles are named as hallmarks of the disease, there is a wealth of inconsistent reports relating these as normal features to the clinical expression of AD (e.g. Arriagada, Growdon, Hedleywhyte, & Hyman, 1992; Berg et al., 1998; Giannakopoulos et al., 2003). The discrepancy between the severity of AD-associated neuropathology and its clinical expression has been a source of discussion in the literature (e.g. Nelson, Braak, & Markesbery, 2009; Jagust, 2016).

The connectivity of the neural system is widely thought to be the substrate of cognitive function. A loss on connectivity, through the loss of synapses particularly in the frontal and temporal cortex, is thought to be an early event in AD pathogenesis, and to be the strongest neurobiological correlate of cognitive dysfunction in AD (DeKosky & Scheff, 1990; Terry et al., 1991; Scheff & Price, 2006; Scheff, Price, Schmitt, Dekosky, & Mufson, 2007; Arendt, 2009). Evidence of synaptic dysfunction and loss being closely linked with the clinical expression of AD also gives rise to the idea that the synapse should be the focus of therapeutics. However, given synapse loss is particularly apparent within or surrounding fibrillar Aβ plaques in both human cases and in transgenic mouse models (Masliah et al., 1994; Sze et al., 1997; Hu, Wong, Côté, Bell, & Cuello, 2003; Dong, Martin, Chambers, & Csernansky, 2007), leads to the hypothesis of Aβ being a driving force of synaptic loss and subsequent cognitive dysfunction in AD.

1.1.2 Animal models of Alzheimer's disease

Transgenic animal models that exhibit the cardinal features of AD are at the forefront of insight gained into the disease. A considerable degree of molecular understanding of AD has come from studying familial AD (FAD)-linked mutations,

namely the APP, PS1 genes that encode the amyloid precursor protein and presenilin 1. Many transgenic mouse models used to study the pathogenesis of AD utilize these FAD mutations, which lead to amyloid pathology and memory impairment (Puzzo, Gulisano, Palmeri, & Arancio, 2015). Mouse models with APP and PS1 mutations are considered an early-stage AD model, and do not develop the second major pathological feature of AD, neurofibrillary tangles (Garcia-Alloza et al., 2006).

1.1.3 Amyloid pathology

It is widely believed that Aβ is central to the pathogenesis of AD. In this regard, the 'amyloid cascade hypothesis' proposes that AD is initiated by an imbalance between the production and clearance of Aβ (Hardy & Selkoe, 2002). The Aβ peptide is derived from the proteolytic cleavage of APP. APP is processed by non-amyloidogenic or amyloidogenic pathways. APP is delivered to the surface membrane where it is cleaved by α-secretase. APP molecules not cleaved by α-secretase can be internalised into endocytic compartments and cleaved by β-secretase then γ-secretase to generate Aβ (O'Brien & Wong, 2011). The most abundant form of Aβ is the $A\beta_{40}$ species, however, the $A\beta_{42}$ species has a greater propensity to aggregate into the Aβ plaques characteristic of AD (Thal, Walter, Saido, & Fändrich, 2015). There is some contention in the literature on the association between the presence of Aβ neuropathology and the clinical expression of dementia. A study utilising PET-amyloid imaging led to the detection of Aβ deposition correlating with episodic memory decline, one of the main clinical features of AD (Villemagne et al., 2013). However, a meta-analysis including 3000 cognitively normal subjects reported the strength of this relationship to be small (Hedden, Oh, Younger, & Patel, 2013).

1.2 Brain and Cognitive reserve

In the classic study by Katzman et al. (1989), it was reported through post-mortem examination, that some people with AD-like neuropathology had no clinical phenotype of the disease before death. This pioneering finding led the authors to postulate that neuropathological burden does not necessarily directly correlate with cognitive outcome. In order to explain the possible disconnect between Aβ pathology and clinical expression of the disease, it was hypothesized that there may be a brain reserve that allows one to function at a non-impaired level despite the presence of neuropathology (Satz et al., 1993). The concept of brain reserve is hypothetical, however, brain size and synapse count are thought to encompass the measurable substrate of brain reserve (Satz et al., 1993; Stern, 2002). Katzman et al. (1988) further found individuals with intact cognition despite the presence of substantial AD-associated pathology, also had larger brain weight, offering support that brain reserve offers one capacity to cope with pathology. It is further posited that with disease process progression, brain reserve capacity is depleted, and once this critical point is reached, clinical deficits emerge (Satz, 1993).

The cognitive reserve theory proposes the brain has the capacity to actively cope with pathological damage in order to preserve cognitive function (Stern, 2002). The theory as applied to AD suggests that pathological damage to the brain can be offset by the use of pre-determined cognitive strategies and the efficient use, or differential recruitment, of neural networks (Stern, 2002; 2012). That is, people who engage in higher levels of mental activity develop stronger, more elaborated, and more efficient neuronal networks, allowing them to withstand a greater degree of pathological burden before clinical expression of the disease emerges.

1.3 Can plasticity be promoted to offset dementia?

Plasticity is not only a substrate of cognition, the brain's plastic capacity also allows it to compensate for neuronal insult (Jellinger & Attems, 2013), such as that encountered by AD. Promoting plasticity in ageing may therefore assist the neural system in coping with ageing-related pathological alterations. Epidemiological evidence suggests cognitive activity may act in the promotion of neural plasticity, and have a protective role against dementing conditions.

There is a putative link between education and dementia, an association pioneered by Mortimor (1988; Sharp & Gatz, 2011). Several studies have offered support for this association over subsequent years. In studies of catholic clergy, education was found modulate the expression of dementia due to AD, where those more highly educated withstood a greater amount of neuropathology before AD manifested clinically (Bennett et al., 2003). Further evidence suggests that it is not only formal education that mediates dementia risk, but various components of cognitive lifestyle. A systematic review of 22 longitudinal cohort studies reported a highly cognitively engaged lifestyle, which encompassed high levels of education, occupation, and engagement in other forms of complex cognitive activity, was associated with a 46% lower dementia risk than those who had a minimally active cognitive lifestyle (Valenzuela & Sachdev, 2006). In addition, engagement in leisure and physical activity has been reported to offer protection against ageing-associated cognitive decline (Friedland et al., 2001; Scarmeas, Levy, Tang, Manly, & Stern, 2001; Wang et al., 2013; Kamegaya, Araki, Kigure, & Yamaguchi, 2014; Marioni et al., 2015). Together, this evidence has offered support for a stimulating lifestyle to have a fundamental role in healthy brain ageing.

Based on such epidemiological evidence, there is considerable and growing interest in the capacity of the central nervous system (CNS) to protect against, or compensate for pathological damage of the brain associated with AD. Although the epidemiological evidence is showing promise, the extent to which cognitive activity may build reserve and protect against AD or ageing-associated cognitive decline is difficult to determine from such studies, primarily because the effect of cognitive activity cannot be isolated from various other lifestyle factors. Moreover, the underlying neural mechanisms that may allow for this protection can rarely be investigated in human populations.

1.4 Environmental enrichment

Environmental enrichment (EE) is a paradigm applied to animal models, in order to experimentally model the promotion of neural plasticity from experience. The paradigm involves the manipulation of an animals' environment, aimed at encouraging environmental stimulation with objects designed to generate physical, sensory, and cognitive activity. The paradigm is used to study how experience from the environment influences the development, refinement, and maintenance of the neural system. The earliest studies starting in the 1960's reported changes in the neuroanatomy of the healthy rodent brain following exposure to EE, including increased cortical thickness (van Praag, Kempermann, & Gage, 2000). Heightened brain connectivity and markers of plasticity were subsequently reported, in which increased dendritic branching, spine density, and synaptic contacts have been observed in EE exposed rodents (Greenough, Volkmar, & Juraska, 1973; Connor, Wang, & Diamond, 1982; Leggio et al., 2005). Living in a stimulating environment has been demonstrated to promote an array of morphological and molecular changes within the healthy CNS that are thought to

modify behaviour and heighten cognitive function compared to animals housed in standard housing (SH; Segovia, del Arco, & Mora, 2009).

1.4.1 Environmental enrichment as an intervention for AD

Given the range of beneficial effects on the structure and function of the nervous system in healthy animal models, the EE paradigm has been applied to animal models of neuropathology. Transgenic FAD animal models allow for the study of the effect of stimulation from the environment, EE, on the pathological, cognitive, and behavioural alterations observed in the disease.

A potentially pivotal study by Kamenetz et al. (2003) led to the suggestion that cognitive stimulation may affect the biochemistry of AD. In organotypic hippocampal slice cultures from transgenic mice harbouring the APP mutation, neuronal activity was found to bi-directionally control Aβ levels. Blocking neuronal activity by a GABA-A receptor potentiator resulted in a reduction of Aβ levels, whilst inducing neuronal activity with a GABA-A channel blocker, led to an increase in Aβ. The study also demonstrated a modulation of synaptic plasticity following manipulation of neuronal activity. A global down-regulation of excitatory synapses was reported following a decrease in neuronal activity, and an increase in neuronal activity up-regulated excitatory synapses. The finding that neuronal activity can regulate APP processing, and thus Aβ production has led to the hypothesis that cognitive stimulation may affect AD pathology by influencing neuronal proteins implicated in the disease (Wang, Megill, He, Kirkwood, & Lee, 2012).

Following this finding, Jankowsky, Xu, Fromholt, Gonzales, & Borchelt, (2003) proposed to model a functional change in synaptic activity *in-vivo*. In this study, an EE paradigm was employed in order to promote neuronal activity, and the subsequent effects on Aβ pathology in a APP/PS1 mouse model was assessed. Female

mice were placed into EE at an early, pre-symptomatic stage of the disease. Paradoxical to the reported beneficial effects of EE found in healthy rodents, and to that of *in-vitro* studies, the authors reported a higher plaque burden in EE-exposed APP/PS1 mice, yet increased cognitive function. The authors suggested higher levels of cognitive stimulation accelerates amyloid pathology, but compensatory mechanisms brought about by enhanced brain function may mitigate the effects of pathology. Despite the counterintuitive findings, the authors demonstrated that amyloid pathology in an AD mouse model to be altered in response to experience from the environment.

Since the initial study by Jankowsky and colleagues (2003) a number of investigations on the effect of EE on a range of transgenic AD mouse models have been performed. Arendash et al. (2004) approached the enrichment paradigm for an APP mouse model therapeutically by starting EE at an advanced stage of AD pathology, rather than preventatively, or before the onset of Aβ pathology, as did Jankowsky and co-workers (2003). Mice with the human APP mutation were entered into EE at an advanced stage of AD at 16 months of age. These mice lived in a constantly enriched environment for four months, and were exposed to a novel environment weekly. At 22 months of age, EE mice performed superiorly on a battery of cognitive testing procedures when compared to mice housed in standard housing. However, these cognitive improvements were independent to that of Aβ load. Total Aβ load in the parietal cortex and hippocampus were of a moderate level regardless of housing condition. The authors concluded in partial agreement with Jankowsky et al. (2003), that the behavioural benefits of EE to AD may involve a separate mechanism to that of abnormal Aβ processing and deposition. The findings of the two studies highlight the ambiguous association of Aβ deposition and the clinical expression of the disease.

Lazarov et al. (2005) hypothesised a decrease in Aβ pathology could occur in response to the environment, if an enriched "experience" was introduced in early life. At weaning, male APP/PS1 mice were exposed to EE for five months. Lazarov and colleagues observed a dramatic reduction in Aβ deposition in the neocortex and hippocampus of EE mice compared to that of SH mice. One particularly noteworthy finding of the study was that Aβ burden was inversely correlated with physical activity (time spent on the running wheel). Lazarov et al. further demonstrated that the activity of the Aβ degrading enzyme, neprilysin, was elevated in the brains of EE AD mice compared to WT, and AD SH mice. The authors suggested their remarkably different findings from that of Jankowsky and co-workers (2003) could be due to sex of the subjects, as female mice were employed for this study, which have a higher amyloid burden than age-matched males (Wang, Tanila, Puoliväli, Kadish, & Van Groen, 2003). However, Arendash and colleagues (2004) employed male APP mice, albeit at an older age, and did not demonstrate a reduction in pathology. Although conflicting with the previous *in-vivo* studies, Lazarov et al. provided the first experimental evidence that exposure to EE reduces Aβ burden.

Jankowsky et al. (2005) further investigated the effect of EE in female APP/PS1 mice by housing mice in the EE condition from two months to eight months of age. At eight months, cognitive testing revealed EE mice to have significantly better spatial memory that SH mice. However, EE mice had a 25% higher Aβ load in comparison to SH mice, a result consistent with the earlier 2003 study. The authors suggested a sexually dimorphic response to EE might be the reason behind the different pathological outcomes to that of Lazarov and co-workers (2005). Moreover, the authors put forward that multiple studies have documented human patients to have intact cognitive function, despite the presence of Aβ plaques at a level sufficient for an AD

diagnosis. Thus, the authors contribute their results to EE working through a mechanism independent to Aβ deposition, such as through building cognitive reserve, and conclude that the nervous system can be substantially influenced by the environment.

Costa et al. (2007) extended the work from Arendash et al. (2004) to determine if EE not only worked as a therapeutic against AD-associated cognitive dysfunction, but whether it could protect against cognitive impairment. At weaning, male and female APP/PS1 mice were placed into EE or SH for 4.5-5.5 months. Following, half of the mice underwent comprehensive behavioural testing, while the other half did not, in order to control for the potential enriching effect that behavioural testing may have. Enriched mice outperformed SH mice, and there were no differences in performance between transgenic EE and healthy wildtype SH mice. The authors suggested that EE allowed for cognitive protection in the transgenic AD mice, bringing their cognitive function up to the level of the healthy control mice. While non-behaviourally tested mice showed no difference in Aβ levels, behaviourally tested EE mice demonstrated lower Aβ deposition than non-behaviourally tested mice. The authors suggested consistent with the findings of Lazarov et al. (2005) that a more demanding EE paradigm, that is EE in combination with behavioural testing, must induce brain changes that compensate for the damage brought about by Aβ pathology.

1.4.2 What type of EE paradigm is efficacious in FAD mouse models?

EE paradigms used for FAD animal models do encompass potential confounders, such as the use of different FAD models, age and stage of pathology, gender, type of EE paradigm, and length of exposure to EE, whether EE is intermittent or stable, or includes a novelty component, that might explain the variable findings

regarding Aβ burden. The discrepancy in findings on the effect of EE on amyloid pathology (Table 1) may be due to the type and intensity of the intervention, as this is not controlled for in such studies. The study raises implications for protecting against AD in humans, with evidence provided for a combination of stimulating activities offering the greatest protection against AD.

As also demonstrated in Table 1, EE is introduced at different ages in various studies, and importantly with varying stages of pathology. While previous investigations focused on starting enrichment at an early, pre-symptomatic age and lasting over disease progression, Herring et al. (2011) set out to examine the effects of living in EE only before, or after AD onset. Female mice harbouring the APP mutation were entered into EE either prior to disease onset and entered back into standard cages thereafter, or lived in SH until disease onset and the transferred to EE. Both preventive and therapeutic strategies reduced total Aβ plaque area in these mice. The authors concluded that stimulation delivered either before or after disease onset can reduce Aβ pathology.

Verret et al. (2013) reported a reduction in Aβ burden following EE that started in early life in a FAD mouse model. However, mice exposed to EE later in life and following the deposition of Aβ, the intervention had no effect on Aβ load. These findings might suggest that an intervention initiated before the onset of amyloid-deposition may slow amyloidosis, however, once pathology has progressed, EE has little effect on Aβ disease process progression.

As AD is a 'silent' disease for many years before clinical expression and subsequent diagnosis (Price & Morris, 1999; Morris et al., 2001), it is important to

investigate interventions targeted at late-life prevention of dementia when an intervention is more likely to be taken up, or to act therapeutically in people living with dementia when it is clear intervention is warranted. Although evidence suggests that after disease onset it is too late to alter Aβ pathology (Verret et al., 2013), there is evidence to suggest that cognitive protection may be afforded by EE by amyloid-independent mechanisms.

1.4.3 Stress as a potential confounder

There is evidence of an association between stress and Aβ pathology, which may play a role in confounding the link between EE and Aβ. The finding of exacerbated Aβ pathology by Jankowsky et al. (2003; 2005) has been suggested to be a result of the EE paradigm inducing stress. These animals did have improved cognitive function despite increased Aβ burden. There is some evidence that mild stressors result in greater capacity of resource management in challenging environmental conditions (Crofton, Zhang, & Green, 2015). The idea that such a stressor not only exerts negative effects, may explain the finding of superior cognitive function and exacerbated Aβ pathology in mice reared in EE. In contrast, Jeong et al. (2011) exposed FAD mice to a stress and EE paradigm, and found that EE counteracted the negative effects of stress on AD disease process progression. Given the highly variable findings of EE on Aβ pathology, and evidence that stress plays a major role in disease onset and progression, it is important to investigate the effects of heightened stimulation on stress, and consider how it may moderate the relationship between EE, cognition, and pathology.

Experiencing a stressful stimulus induces activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to a release of glucocorticoids from the adrenal cortex. Evidence of HPA axis dysfunction in AD comes from the finding that people

living with AD typically have increased blood levels of the stress hormone cortisol (Rasmuson et al., 2001; Csernansky et al., 2006). Elevated levels of the glucocorticoid (corticosterone in rodents) has also been reported in a FAD mouse model (Guo, Zheng, & Justice, 2012). However, whether chronic stress and subsequent elevated cortisol increases risk of developing AD, or whether increased cortisol is a symptom of AD, is unknown.

The mechanisms underlying the finding of an association between elevated stress hormone and AD have been explored in FAD rodent models. Green, Billings, Roozendaal, McGaugh, and LaFerla (2006) reported in a FAD mouse model, that Aβ pathology preceded HPA axis dysfunction. Green and colleagues speculated damage to the hippocampus by Aβ plaques would lead to disinhibition of the HPA axis. Disinhibition of the HPA axis would lead to an abnormal stress response to aversive stimuli, and a subsequent increase in corticosterone. The increasing levels of glucocorticoid may further accelerate pathological processes, acting as a cycle of disease process progression. Alternatively, it is argued that HPA axis dysfunction precedes the formation of extracellular plaques (Rothman et al., 2012). Rothman and co-workers found an increase in Aβ oligomers following a 6-week chronic stress paradigm in a FAD mouse model. FAD mice not exposed to the stress paradigm also had increased basal corticosterone levels despite lower levels of Aβ relative to mice exposed to stress. These data suggest HPA axis dysfunction precedes Aβ plaque pathology, which may suggest that chronic stress and increased cortisol is associated with the development of AD. Whether elevated glucocorticoids precede Aβ pathology, or whether it is a symptom of pathology, the effect of EE on stress is a confounding factor that is seldom investigated.

1.4.4 EE and amyloid-independent cognitive protection

Studies focused on the association between EE and amyloid pathology have also led to findings regarding amyloid-independent effects. Arendash et al. (2004) reported global cognitive protection in ageing FAD mice exposed to EE, despite no reductions in Aβ deposition. Similarly, Jankowsky et al. (2005) reported a counterintuitive increase in Aβ pathology, yet a marked attenuation of cognitive dysfunction in FAD mice exposed to EE. These findings together might suggest EE may not directly exert its affects by mitigating Aβ pathology. EE may build cognitive reserve capacity, allowing for cognitive protection despite pathological brain lesions. EE may exert its effects through alternative neural mechanisms, that are currently elusive. An understanding of these underlying neural mechanisms will likely be invaluable in developing therapeutics to delay or halt the onset of dementia.

1.4.5 EE as a means of promoting synaptic plasticity in AD

Synapse loss is currently the strongest neurobiological correlate of cognitive dysfunction in AD (DeKosky & Scheff, 1990; Terry et al., 1991; Scheff & Price, 2006; Scheff, Price, Schmitt, Dekosky, & Mufson, 2007; Arendt, 2009). Evidence of synaptic dysfunction and loss being closely linked with the clinical expression of AD also gives rise to the idea that the synapse should be the focus of therapeutics. AD is insidious, yet a slowly progressing disease, emerging clinically many years after disease process inception (Price & Morris, 1999; Morris et al., 2001). It is conceivable that the process of synaptic degeneration is also a slow-acting process, and there may be a point at which the degeneration of synapses can be rescued. Concurrently, the nervous system has remarkable capacity for plasticity; axons can reinnervate CNS sites and create new synapses (Coleman, Federoff, & Kurlan, 2004). The plasticity of the synaptic system

might be drawn upon when the brain is infested by AD-related neuropathology to stave off, or slow the progression of cognitive dysfunction. While synaptic plasticity is perturbed in AD (Chapman et al., 1999; Ma & Klann, 2012; Sorrentino, Iuliano, Polverino, Jacini, & Sorrentino, 2014), stimulation from the environment may create heightened capacity for plasticity and subsequent compensation for pathological damage allowing for cognitive maintenance.

A number of studies performed on healthy wildtype rodents have demonstrated a spectrum of synaptic alterations following EE. Postsynaptic changes have been observed through the analysis of dendritic spines in the cerebral cortex and hippocampus, in which spine densities were increased by EE (e.g. Turner & Greenough, 1985; Rampon et al., 2000; Kolb, Gibb, & Gorny, 2003; Gelfo, De Bartolo, Giovine, Petrosini, & Leggio, 2009). Jung and Herms (2014) using *in vivo* microscopy, demonstrated increases in density and turnover of dendritic spines in enriched mice. Presynaptic alterations following EE have also been reported in healthy animals (Nithianantharajah, Levis, & Murphy, 2004). However, investigations on synaptic alterations following EE in AD mouse models have been more limited. Levi, Jongen-Relo, Feldon, Roses, & Michaelson, (2003) investigated EE-induced effects on mice containing the APOE E3 or E4 allele. APOE E4 is a risk factor polymorphism for AD (Verghese, Castellano, & Holtzman, 2011), and animals with this genotype did not show cognitive improvement following EE. Moreover, the E3 carrier mice showed not only heightened cognitive function compared to those carrying E4, but also increased levels of hippocampal nerve growth factor (NGF) and heightened levels of synaptophysin.

Further evidence of EE increasing plasticity was provided by Costa et al. (2007). Superior cognitive function was reported in an APP/PS1 mouse model exposed

to long-term EE. Dendritic branching defects observed in these transgenic animals were not overcome by EE, however, EE was found to induce beneficial changes in gene expression related to neuronal plasticity. The authors suggested rather than EE directly targeting Aβ pathology, more subtle changes, such as alterations in neuronal plasticity-related gene expression may offer an explanation for the finding of heightened cognitive function in the absence of a reduction in Aβ pathological burden. Herring et al. (2011) also provided evidence for EE up-regulating expression of plasticity-associated proteins in an APP mouse model. The authors demonstrated transgenic AD mice living in an EE to have increased levels of the plasticity-associated protein Arc and heightened synaptic density brought up to the level of healthy wildtypes, and significantly higher than transgenic mice living in SH. In addition, Lazarov and colleagues (2005) demonstrated through microarray analysis, a number of genes differentially regulated by EE in a FAD mouse model, with elevated expression of genes involved in neurogenesis and neuronal plasticity.

Catlow et al. (2009), and Mirochnic, Wolf, Staufenbiel, & Kempermann (2009) investigated whether the cognitive protection observed in rodents that underwent EE in previous studies, was afforded by an increase in neurogenesis. Mirochnic et al. compared APP mice that lived in either SH, EE, or physical activity housing for adult neurogenesis in the hippocampal dentate gyrus at 6 and 18 months. At the 18-month time-point, mice living in SH had a higher $A\beta_{1-42}$/ $A\beta_{1-40}$ ratio as well as a lower number of newborn granule cells in the hippocampus dentate gyrus compared to the EE and physical activity housing conditions. Catlow et al. however, also investigated neurogenesis in the hippocampal dentate gyrus in APP/PS1 mice and found no difference among impoverished, EE, or physical stimulation housing conditions. The authors concluded that the generation of new neurons in the hippocampal dentate gyrus

is not involved with cognitive protection following EE. Mirochnic and colleagues' findings on the other hand suggested that enrichment is effective in increasing adult hippocampal neurogenesis, and perhaps aids in protecting cognitive function.

1.4.6 EE, AD, and brain-derived neurotrophic factor

Alterations in neurotrophins, such as brain-derived neurotrophic factor (BDNF), may provide one underlying mechanism of the association between EE and cognitive benefit in FAD models. BDNF is a nerve growth factor expressed throughout the CNS, and is vital for the maintenance, survival and growth of neurons (Mattson, Maudsley, & Martin, 2004). In addition, BDNF promotes neuronal survival, neurite growth, and synthesis of neurotransmitters and neurogenesis (Barde, Edgar, & Thoenen, 1982). Considerable evidence from *in vitro* and *in vivo* experimental studies suggests BDNF to have pro-survival functions on neurons under different pathological conditions (Lu, Nagappan, Guan, Nathan, & Wren, 2013), signifying BDNF may be a key target molecule in developing therapeutics for various diseases of neuropathology.

BDNF has been implicated in AD, with reduced levels of BDNF found in the hippocampus and frontal and parietal cortices of the AD brain (Ferrer et al., 1999; Hock, Heese, Hulette, Rosenberg, & Otten, 2000). As BDNF mediates synaptic plasticity and cognitive function (e.g. Murer, Yan, & Raisman-Vozari, 2001; Lu, 2003; Yoshii & Constantine-Paton, 2010), it is thought that BDNF may be critically involved in the pathophysiology underlying cognitive decline in AD. BDNF regulates processing of APP through the non-amyloidogenic pathway (Fu, Lu, & Mattson, 2002; Rohe et al., 2009), which may yield beneficial effects, such as a reduction in the production of Aβ peptides, and the release of the secreted form of APP, which is associated with neuroprotective effects (Scheuner et al., 1996; Nishitomi et al., 2006; Thornton, Vink,

Blumbergs, & Van Den Heuvel, 2006). Moreover, studies employing *in vitro* methods have reported a protective effect of BDNF on $A\beta_{42}$ (Arancibia et al., 2008; Aliaga et al., 2010).

Additional evidence that BDNF expression should be promoted in AD comes from the increasing view that AD is a disease of synaptic plasticity failure. BDNF promotes synapse formation (Park & Poo, 2013), which may be through the promotion of dendritic growth (McAllister, Lo, & Katz, 1995), axonal branching (Cohen-Cory & Fraser, 1995) and activity-dependent synapse refinement (Cabelli, Hohn, & Shatz, 1995). Due to evidence of the beneficial effects of BDNF on synaptic plasticity, it is also thought to play a critical role in cognitive function. Despite the accumulating evidence that therapeutic strategies should target BDNF pharmacologically, to date BDNF has not been able to be delivered across the blood-brain barrier (Lu et al., 2013). However, some evidence suggests EE may be able to increase endogenous levels of BDNF.

Increases in BDNF expression in the hippocampus of healthy animals following EE have been found (Kuzumaki et al., 2011; Ramírez-Rodríguez et al., 2014; Novkovic, Mittmann, & Manahan-Vaughan, 2015), as well as global increases (Ickes et al., 2000). There has been limited work on the relationship between EE and BDNF in FAD models. However, Wolf and co-workers (2006) reported in an APP reported improved cognitive function following EE, possibly accounted for by an up-regulation of BDNF. There is a wealth of evidence linking increased BDNF expression to physical activity (Erickson, Miller, & Roecklein, 2012), which forms a caveat to the EE research in which it is uncertain if it the physical activity along, rather than cognitive stimulation, that is driving the increased in BDNF.

1.4.7 EE, AD, and microglia

Microglia are the brain's tissue macrophages, the primary immune effector cells in the CNS. Microglia are distributed throughout the brain (Perry, Hume, & Gordon, 1985; Nimmerjahn, Kirchhoff, & Helmchen, 2005) and constantly survey their environment for pathogens, apoptotic cells, and foreign material (Streit, Mrak, & Griffin, 2004). Microglia are critically involved in generating chronic, and self-sustaining neuroinflammation (Rubio-Perez & Morillas-Ruiz, 2012), and current evidence suggests neuroinflammation to be key contributor to the pathogenesis of AD (Heneka et al., 2015). Evidence suggests that the involvement of neuroinflammation to be an early, and substantial process leading to AD pathogenesis, further supported by the finding that genes for immune receptors are associated with AD (Bradshaw et al., 2013; Griciuc et al., 2013; Guerreiro et al., 2013).

Originally described by Alzheimer over 100 years ago, there is a characteristic increase in microglial activation within the AD brain (Steltzmann, Schitzlein, & Murtagh, 1907; as cited in Mosher & Wyss-Coray, 2014). However, it has only been more recently that the resident macrophages of the brain have received attention for their role in CNS function and dysfunction (Derecki, Katzmarski, Kipnis, & Meyer-Luehmann, 2014). Microglia frame dense-core Aβ plaques, their recruitment to plaques causes a morphologic change, typically thickened, dystrophic processes and increased soma size (Itagaki, McGeer, Akiyama, Zhu, & Selkoe, 1989). In the AD brain, microglia surrounding amyloid plaques, are observed to have an 'activated', pro-inflammatory phenotype (Perlmutter, Barron, & Chui, 1990). Intriguingly, microglia do not surround diffuse Aβ plaques in the "normal" ageing brain (Itagaki et al., 1989; Heurtaux et al., 2010; von Bernhardi, 2007).

Due to frequent observations of highly reactive, phagocytic microglia surrounding Aβ plaques, there are current, extensive research efforts focused on the role of microglia in AD pathogenesis. *In vivo* imaging studies have provided evidence of microglia being recruited rapidly to newly formed Aβ plaques, and microglia processes remain highly dynamic (Meyer-Luehmann et al., 2008). It is thought that microglia surrounding Aβ plaques appear to form a barrier that restricts plaque growth, in order to prevent the diffusion of Aβ oligomers that are synaptotoxic (Bolmont et al., 2008; Meyer-Luehmann et al., 2008; Condello, Yuan, Schain, & Grutzendler, 2015). However, microglia may become dysfunctional after they are recruited to Aβ plaques (Krabbe et al., 2013). There is evidence of microglial dysfunction in ageing, which may mean microglia are unable to form an effective barrier surrounding plaques. During ageing, microglia undergo morphological alterations; they show an increased soma size, and processes are shorter, sparse, and dystrophic (Streit, 2006; Flanary, Sammons, Nguyen, Walker, & Streit, 2007). These morphological alterations are thought to confer functional deficits, as observed in *in vivo* and *in vitro* experiments, microglia in ageing may be unable to phagocytose Aβ effectively (Floden & Combs, 2011; Sheng, Mrak, & Griffin, 1998; Sierra, Gottfried-Blackmore, Mcewen, & Bulloch, 2007), potentially providing one explanation for AD being more generally a disease of ageing.

As discussed previously, AD is a disease of synaptic loss and synaptic plasticity failure (DeKosky & Scheff, 1990; Terry et al., 1991; Scheff & Price, 2006; Scheff et al., 2007; Arendt, 2009). There is emerging evidence of microglia being critically involved in the elimination and refinement of synaptic connections in the healthy brain. Microglial processes constantly contact axons, dendritic spines, and synapses (Stevens et al., 2007; Paolicelli et al., 2011; Schafer et al., 2012). The dynamics of microglial processes have been found to be regulated by sensory experience and neuronal activity

(Wake, Moorhouse, Jinno, Kohsaka, & Nabekura, 2009; Tremblay, Lowery, & Majewska, 2010), and have also been implicated in learning processes, through their interaction with synaptic contacts. A study where microglia were ablated in the adult rodent brain, led to an impairment in learning, and an associated reduction in learning-induced synapse formation in the motor cortex (Parkhurst et al., 2013). These findings together suggest microglia to have a role in experience-dependent synaptic plasticity.

Recent evidence suggests that lifestyle factors such as physical and cognitive activity can play a role in promoting microglial health in ageing. In the APP/PS1 FAD mouse model, physical exercise was associated with a reduction in both Aβ levels and microglial activation (Ke, Huang, Liang, & Hsieh-Li, 2011). There have also been reports that the neuronal benefits conferred by EE may occur by modulation of innate immune cells, including microglia (Ziv et al., 2006; Choi et al., 2008; Vukovic, Colditz, Blackmore, Ruitenberg, & Bartlett, 2012). Moreover, an association between EE and the quantity of microglia occupying neocortex and hippocampus has been reported (Ehninger & Kempermann, 2003; Williamson, Chao, & Bilbo, 2012; Xu et al., 2016). There has been limited research conducted on whether EE has the potential to modify microglia and promote microglial health in AD. However, one recent study investigated this potential link by examining how EE might regulate microglial function in healthy Wt mice injected with oligomeric Aβ. Xu et al. (2016) found EE to suppress the elevated expression of inflammatory genes that occur in response to oligomeric Aβ, and a decrease in uptake of oligomeric Aβ by microglia. The decrease in Aβ phagocytosis was hypothesised to be due to the decrease in pro-inflammatory reaction by EE mice. Overall, the authors reported EE to modulate microglial morphology and density, leading to an increase in microglial resistance to the effects of oligomeric Aβ.

The study by Xu and colleagues (2016) offered significant insight into one of the underlying mechanisms of the protective effect of EE, and suggested EE could protect against AD through modulating the brain's innate immune system. However, in this study, relatively young mice (approximately 3 months) were employed. It is during ageing that microglia are hypothesised to be less efficient at removing Aβ, and through the later stages of AD, perhaps become completely dysfunctional (Hickman, Allison, & El Khoury, 2008; Lee & Landreth, 2010). How EE might modulate the brain's immune system in ageing, and through disease progression, remains unknown.

Table 1. EE interventions for models of amyloid pathology

First Author (publication year)	FAD mouse model	*n*	Sex	Age entered into EE (months)	Duration of EE	Type of EE	Cognitive function	Aβ pathology
Jankowsky (2003)	APPswe, $PSEN1_{dE9}$	32	F	2	6	Constant with objects rearranged weekly	↑	↑
Arendash (2004)	APP_{SWE}	13	Not stated	16	3	Intermittent (3 x week)	↑	-
Lazarov (2005)	APPswe, $PSEN1_{dE9}$	13	M	1	5	First month constant (3 hrs/day), then 3 x week	Not assessed	↓
Jankowsky (2005)	APPswe, $PSEN1_{dE9}$	76	F	2	6	Constant	Not assessed	↑
Costa (2007)	$APP^{+/-}$ $PS1^{+/-}$	101	M + F	1	6	Constant + 3 x week novel exposure	↑	↓
Cracchiolo (2007)	$APP_{SWE,}$ $APP_{SWE}PS1_{M67IL}$	38	M + F	1.5	7	Social/Physical/ Complete + (novelty exposure 3 x week)	↑	↓
Mirochnic (2009)	APP23	38	F	6 & 18	4	Constant with objects rearranged	Not assessed	-
Herring (2011)	APP695	38	F	1	5	Constant	Not assessed	↓
Valero (2011)	APP_{SWE}	58	F	4	1.5	Constant with objects rearranged weekly	↑	-
Cotel (2012)	APP/PS1 KI	60	F	2	4	Constant with objects rearranged weekly	-	-
Verret (2013)	APP695	112	F	3, 5, 10	2.5	Constant with objects rearranged	↑	Minor ↓

↑ = EE increased relative to SH; ↓ = EE decreased relative to SH; - = no difference between SH and EE

1.5 Project Aims

Searching for experimental approaches to promote brain plasticity is exceedingly relevant to our society in which ageing-related diseases are becoming increasingly prominent. Strategies aimed at boosting plasticity, or cognitive reserve, offer a non-invasive alternative, and avoids dangerous side effects typical of many pharmacological therapies. Moreover, epidemiological and experimental evidence demonstrates the great potential cognitive interventions may have in preventing ageing-associated cognitive dysfunction. The encouraging data produced from animal enrichment studies offers hope for transferring these results to both human health and disease applications.

While the animal-based literature on EE for healthy animals has a long history, and offers promise in staving off neurodegenerative disease, there is much to be elucidated. EE applied to FAD models has been focused on its effect on amyloid pathology, and findings have been highly variable. The literature also demonstrates a need for increased research on later-life interventions. While there is a wealth of literature supporting early-life cognitive intervention reducing dementia risk, in reality, people will be more likely to take up an intervention when dementia is more relevant to them, during ageing. In addition, as cognitive deficits emerge many years after disease processes start, therapies, in all likelihood, will be introduced following the onset of disease-induced pathology. Whether enrichment can exert similar beneficial effects in ageing as it does in early-life, is still a relatively unanswered question. Moreover, there is limited research on the efficacy of an intervention after disease processes are initiated. The effects of EE on Aβ pathology have been demonstrated to be contentious, and potential moderating factors such as how EE affects the brain's immune cells, synaptic health, and stress hormones have received limited attention.

1.5.1 Aim 1: Investigate the effect of mid-life environmental enrichment on cognitive and synaptic health in healthy and Alzheimer's disease-associated pathological ageing

Early-life cognitive enrichment may reduce the risk of experiencing cognitive deterioration and dementia in later-life. However, an intervention to prevent or delay dementia is likely to be taken up in mid to later-life. Moreover, previous studies have demonstrated the effect of EE on amyloid (Aβ) pathology to be variable, with the effect of EE on synaptic connectivity receiving limited attention. Hence, the aim of this study was to investigate the effects of environmental enrichment (EE) in wildtype (Wt) mice and in a mouse model of Aβ neuropathology ($APP_{SWE}/PS1_{dE9}$; APP/PS1) from 6 months of age. This transgenic mouse model recapitulates pathology that most closely resembles the initial stages of AD, and Aβ deposits have been observed from 4-6 months of age in this model, with abundant plaques present by 9 months (Jankowsky et al. 2004; Garcia-Alloza et al. 2006; Vickers et al. 2009). After 6 months of housing in standard laboratory cages (minimal stimulation), APP/PS1 and healthy Wt mice were randomly assigned to either enriched (EE) or standard housing (SH). Following the 6-month differential housing period, cognitive function was tested in order to determine whether EE initiated in mid-life, and after Aβ plaque deposition, was associated with heightened cognitive function as has been reported for early-life EE. Next, Aβ plaque burden, synaptic, BDNF protein levels were analysed, followed by analysis of synaptic density in the regions Fr2, CA1, and CA3.

1.5.2 Aim 2: Examine the effect of complex, novel environmental enrichment on Aβ neuropathology

The literature surrounding the effect that EE has on Aβ neuropathology is variable, however, there is some evidence that more complex and novel enrichment may be required in order to buffer AD-related neuropathology. The aim of this study was to examine whether there may be a 'dose' effect of EE, with added periodic augmentation of stimulation (EE+) in FAD animals from mid-life (6 months of age). In order to examine dose-effects of EE in mid to later-life, mice were raised in SH conditions until 6 months, and then randomly assigned to SH, EE or enhanced EE (EE+) conditions, where the animals remained until 12 months of age. Moreover, some evidence suggests that EE might be associated with lowered stress, which might explain a reduction in Aβ burden following EE in some studies. Following the differential housing period, stress hormone levels (corticosterone), Aβ plaque load and levels of $A\beta_{42}$ were examined in neocortex and hippocampus.

1.5.3 Aim 3: Explore the effect of late-life environmental enrichment on cognitive function and microglia

Ageing-related alterations to microglia are increasingly recognised as a vital contributor to AD pathogenesis. EE from early life has been robustly associated with neural and cognitive protection, and more recently the brain's immune system has been demonstrated to be vital in this link. However, there has been limited work on how EE potentially effects the brain's immune cells, such as microglia, in pathological and non-pathological ageing. The aim of this study was to assess whether EE initiated in later life, and at a stage of advanced Aβ pathology, would lead to positive cognitive effects as demonstrated in the mid-life cohort. In regions important for learning and memory processes, the neocortex and hippocampus, the area occupied by microglia was examined and compared between groups, and correlated with cognitive measures.

Chapter 2

2. *Materials and Methods*

2.1 Mice

Animals were housed in standard conditions (and environmental enrichment as described below), comprising *ad libitum* access to food and water, and housed at 20°C, on a 12/12-hour light/dark cycle. All experimental procedures were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and approved by the University of Tasmania Animal Ethics Committee (A13253).

2.1.1 Transgenic mouse model of Alzheimer's disease

Male transgenic mice expressing chimeric mouse/human amyloid precursor protein (APP) and mutant human presenilin 1 (PS1) on C57BL/6 background [B6.Cg-Tg (APPswe, $PSEN1_{dE9}$) 85Dbo/J] (APP/PS1; Jankowsky et al. 2004) were used for the present study. This transgenic mouse model recapitulates pathology that most closely resembles the initial stages of AD, and Aβ deposits have been observed from 4-6 months of age in this model, with abundant plaques present by 9 months (Jankowsky et al. 2004; Garcia-Alloza et al. 2006; Vickers et al. 2009).

2.2 Genotyping

Tail clippings were sampled from mice at weaning, and in order to extract genomic DNA, the tail clippings were incubated in 45μl of extraction solution (QUANTA Biosciences) for 30 minutes at 95°C. APP/PS1 genotypes were distinguished from Wt mice by standard polymerase chain reaction (PCR) using MyTaq Red Mix (Bioline) and primers (GeneWorks) for the PS1 transgene, and IL-2 was included as an internal control. Resulting PCR products were electrophoresed on a 2%

agarose gel (Bioline) with 0.0001% SYBR safe DNA gel stain (Invitrogen) at 120V for 35 minutes. The subsequent bands present at 608 bp (PS1) and 324 bp (IL-2) were visualised using a Carestream 4000MM Pro image station.

2.3 Mid-life environmental enrichment paradigm

All animals lived in standard housing (SH) conditions comprising group housing of 4-5 mice per 30 x 30 x 14 cm cage, *ad Libitum* access to food and water, an igloo, one small wooden stick and one tissue from weaning until 6 months of age. $APP_{swe}PSEN1_{dE9}$ (APP/PS1; $n = 27$) and littermate wildtype control (Wt; $n = 21$) mice were randomly assigned to SH or EE conditions at 6 months of age, for the following 6 months. The EE housing condition consisted of a larger 60 x 30 x 14 cm cage with the contents of the SH cage and the addition of enrichment objects (wooden and plastic blocks of differing shapes and sizes, platforms, a ball, running wheel, and a mouse hut). Housing conditions were maintained until the 12-month end-point.

2.3.1 Mid-life environmental complexity and novelty paradigm

All animals lived in standard housing (SH) conditions comprising group housing of 4-5 mice per 30 x 30 x 14 cm cage, *ad Libitum* access to food and water, an igloo, one small wooden stick and one tissue from weaning until 6 months of age. At 6 months, $APP_{swe}PSEN1_{dE9}$ (APP/PS1; $n = 40$) and littermate wildtype control (Wt; $n = 31$) mice were entered into the environmental enrichment plus complexity and novelty condition (EE+). The EE+ condition involved the mice being exposed to a larger (36 x 49 x 22 cm) cage three times per week for a three-hour period during the dark cycle. The EE+ cage contained novel enrichment objects that were different to the EE cage (e.g. steps, ladder, tunnels, mazes, objects of differing textures, a large running wheel,

see-saw, platforms, chew-toys). Housing conditions were maintained until the 12-month end-point.

2.3.2 Late-life environmental enrichment paradigm

All animals lived in standard housing (SH) conditions comprising group housing of 4-5 mice per 30 x 30 x 14 cm cage, *ad Libitum* access to food and water, an igloo, one small wooden stick and one tissue from weaning until 12 months of age. $APP_{swe}PSEN1_{dE9}$ (APP/PS1; $n = 15$) and littermate wildtype control (Wt; $n = 17$) mice were randomly assigned to SH or EE conditions (as in mid-life EE paradigm) at 12 months of age, for the following 6 months. Housing conditions were maintained until the 18-month end-point.

2.4 Behavioural and cognitive assessment

Following arrival, mice were allowed to acclimatize for 7 days prior to any testing. Animals were handled daily and habituated to the test room during the acclimatization period to minimize the effects of stress due to handling at time of testing. All cognitive testing procedures occurred at the same stage of the light/dark cycle for each test. Black curtains surrounded the test equipment whilst mice were tested in order to prevent the mice becoming distracted, or from using extra-maze cues. Testing equipment was wiped down with 70% ethanol between each trial in order to prevent mice using odour cues. The experimenter was blind to genotype of animals. The test room was kept quiet during test periods, and test room lighting was kept constant throughout all testing. All testing was recorded using a JVC digital camera mounted to the ceiling for later analysis. All behaviour testing was manually scored

from the recorded videos, and later validated with the automated tracking software, EthoVision XT.

2.4.1 Y Maze spatial short-term memory

Hippocampal-dependent spatial short-term recognition memory was assessed by the Y maze, a maze consisting of three arms placed 120° apart, with a black and white visual cue at the end of each arm. The two-trial Y maze task is a test based on the innate tendency of rodents to explore novel environments (Dellu, Contarino, Simon, Koob, & Gold, 2000; Wang, Ma, & van den Buuse, 2006). Mice were tested at 6 or 12-month baseline, after 3 months of differential housing, and at completion of the 6-month differential housing period. All mice underwent an initial training trial, where a randomly assigned arm of the Y-maze was blocked (novel arm). Mice were randomly designated to and placed in a start arm facing the wall of the arm, and were allowed to explore the two open arms for 10 minutes. Following training, mice were returned to their home cage for the 1 hour inter-trial interval period. The testing phase began one hour after the training phase. Mice were entered into the designated start arm as in training, and were able to freely explore all arms of maze including the previously blocked novel arm. Mice were allowed to explore the Y-maze for 5 minutes after leaving the start arm. Exclusion criteria applied were failing to leave the start arm after 5 minutes, escaping from the Y-maze, or climbing over the arm barrier in the training phase. Y-maze testing was recorded for later analysis.

2.4.2 Barnes maze spatial learning & long-term memory

The apparatus is a white circular platform positioned 60 cm above the ground, that contains 20 holes evenly spaced around the perimeter of the platform. A hidden escape box was positioned underneath one of the holes. Four visual cues were placed on

curtains surrounding the maze at a distance of approximately 50 cm from the perimeter of the platform (Nithianantharajah & Hannan, 2006). Mice were initially habituated to the maze by being placed on the maze and allowed to freely explore for two 5-minute adaptation trials. Twenty-four hours after adaptation, a seven-day training period begin. Training was performed under bright light (approximately 300W) in order to motivate mice to find the hidden escape box. As this is a test of spatial memory, each mouse had the same designated escape position on the maze, however, each mouse was randomly assigned one of four different escape positions to overcome bias. Mice underwent two training trials per day for seven days in which they were initially placed under an opaque start box in the centre of the maze. After 30s, the start box was lifted and the mouse was able to freely explore the platform. The trial ended when the mouse had climbed into the hidden escape, or after 5 minutes had elapsed. After each trial, the maze and escape box were wiped down with 70% ethanol and the maze was rotated to prevent olfactory cues. Fourteen days after the 7-day training period had elapsed, long-term memory was assessed using the same protocol as in training.

2.5 Tissue collection

Following the final day of behavioural testing all mice were weighed to ensure no significant group differences that could confound experiments, and were terminally anaesthetized first with gas anaesthesia (isoflurane) followed by sodium pentobarbitone (100 mg/kg delivered intraperitoneally). For histology, animals were perfused transcardially with 4% paraformaldehyde (PFA) in 0.1M phosphate buffered saline (PBS, pH 7.4). Postmortem brains were transferred to 18% then 30% sucrose solutions overnight. Brains for histology were serially sectioned on a cryostat (Leica CM 1850) in 40μm coronal sections. Sections used for analysis were from bregma 2.0 - ¯3.0 mm according to the stereotaxic mouse brain atlas (Paxinos & Franklin, 2008). For Western

blotting and Enzyme-linked immunosorbent assay (ELISA) procedures, animals were transcardially perfused with PBS (0.1M). Postmortem brains were removed and the cortex and hippocampus were dissected and immediately frozen in liquid nitrogen. Cortex and hippocampal samples were stored at -80°C for later analysis.

2.6 Immunohistochemistry and histological stains

2.6.1 Thioflavin-S staining for Aβ plaques

Thioflavin-S (Sigma-Aldrich) staining was performed in order to visualise fibrillar and dense-core Aβ plaques (Dickson & Vickers, 2001). Ten serial sections evenly spaced throughout the rostrocaudal axis of the brain from bregma 2.0 - ¯3.0 mm according to the stereotaxic mouse brain atlas (Paxinos & Franklin, 2008) were incubated in the solution (0.125% Thioflavin-S diluted in 60% absolute ethanol with 40% 0.01M PBS) for 3 minutes at room temperature, and washed for 2 x 1 minute in 50% absolute ethanol and 50% 0.01M PBS solution, followed by 3 x 10 minute washes in 0.01M PBS. Sections were mounted using Dako fluorescent mounting medium.

2.6.2 MOAB-2 immunohistochemistry for Aβ plaques

For the quantitation of Aβ deposits, antigen retrieval by formic acid treatment was performed in order to enhance immunoreactivity for the visualization of Aβ deposits (Kai et al., 2012). Ten sections evenly spaced across the rostrocaudal extent of the brain (bregma 2.0 - ¯3.0 mm) were incubated in 88% formic acid (Sigma-Aldrich) at room temperature for 8 minutes, followed by 6 x 10 minute PBS washes. Free-floating sections were washed for 3 x 10 minutes in 0.25% Triton-X-100 and incubated with serum-free protein block (Dako) for 15 minutes at room temperature. The sections were immunolabelled with the MOAB-2 antibody (1:2000; Novus Biologicals, Table 2.1)

which specifically labels mouse and human un-aggregated Aβ, following the protocol outlined in Collins, King, Woodhouse, Kirkcaldie and Vickers (2015). MOAB-2 labelling was visualized by incubation in Alexa-fluorophore conjugated secondary antibody (1:1000; Molecular Probes, goat anti-mouse IgG2b-546, Table 2.2) and cover-slipped using Dako fluorescent mounting medium.

2.6.3 Immunohistochemistry for synaptophysin puncta

To determine region-specific alterations in synaptic density, synaptophysin immunoreactive puncta were quantitated histologically in specific areas of the brain including the dorsomedial region of the murine prefrontal cortex, frontal area 2 (Fr2) and the hippocampal subregions CA1 and CA3 (Figure 2.1). Five images across three sections within Fr2 (bregma 1.98 – 0.38 mm) and five images across CA1 and CA3 (bregma ¯1.28 - ¯2.12 mm) were immunostained with primary anti-synaptophysin antibody (1:200; Millipore, Table 2.1) using Alexa-fluorophore secondary antibody, goat-anti-rabbit IgG 594 (Molecular Probes, Table 2.1). To highlight architecture, sections were also incubated with the nuclear stain DAPI (5 µg/mL; Invitrogen) for 5 minutes at room temperature. Sections were mounted using fluorescent mounting medium (Dako).

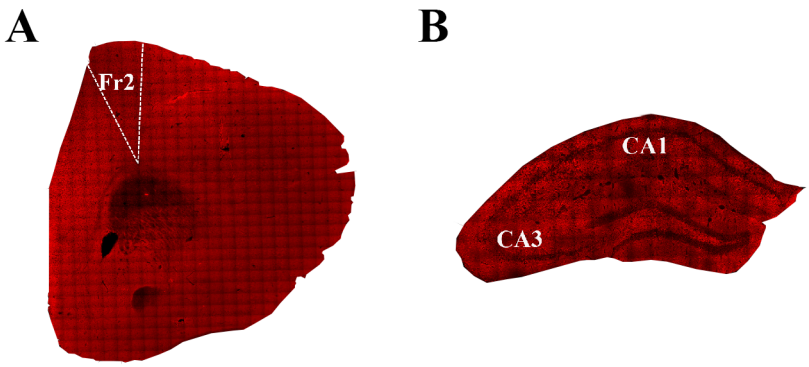

Figure 2.1. Regions selected for synapse analysis. **A** Right hemisphere of coronal mouse brain section displaying Fr2. **B** Image of hippocampus showing CA1 and CA3 subregions.

2.6.4 Immunohistochemistry for microglia

Three sections at approximately bregma 1.045, -1.855, and -2.88 mm were incubated in formic acid as described for MOAB-2, and immunolabelled with the Iba1 antibody (1:1000; Wako, Table 2.1) which recognises the ionized calcium binding adaptor molecule 1, labelling microglia. The Iba1 antibody and the labelling was visualised by incubation with Alexa-fluorophore conjugated secondary antibody (1:1000; Molecular probes, goat anti-mouse IgG2b -546, Table 2.2). The sections were mounted using Dako fluorescent mounting medium.

2.7 Image acquisition

In order to determine Aβ plaque load in the cortex and hippocampus, images were obtained with a Leica DM fluorescence microscope on a 10x objective and NIS Elements imaging software. Ten sections evenly spaced from the rostral to caudal extent of the cerebral cortex from bregma position 2.0 to -3.0 mm were imaged for MOAB-2 and Thioflavin-S Aβ plaque load. The left side of the cortex was imaged from the midline to the rhinal fissure (rf; Figure 2.2). Images of the whole hippocampus were taken between bregma position -1.22 and -2.46 mm of 3-5 sections per animal. Aβ plaque load (defined as percentage area occupied by MOAB-2 immunoreactivity or Thioflavin-S fibrillar staining) was determined by random forest segmentation (O'Mara et al. manuscript in preparation).

Imaging of Synaptophysin and Iba1 labelling was performed on a Perkin-Elmer Ultraview VOX confocal imaging system with Volocity 6.3 imaging software. For synaptophysin imaging, all images were acquired with the same laser power and

exposure settings, using a 60x objective. Five images/section were taken within Fr2, CA1, and CA3 subregions of 3 sections/animal. For APP/PS1 animals, Aβ plaque free regions were included in the analysis. An imageJ watershed algorithm with Gaussian blurring ($\sigma = 1$) was applied and synaptophysin positive puncta were automatically segmented by random forest classification (O'Mara et al. manuscript in preparation) and an ImageJ watershed algorithm was applied to the segmented images. Particles ranging from 0.15 to 2.0 μm^2 were quantified (Stanislaw Mitew, Kirkcaldie, Dickson, & Vickers, 2013). Synaptic density was calculated as the number of Synaptophysin immunolabelled puncta per field, corrected for cell body area. Three sections at bregma positions 1.045, -1.855, and -2.88 mm stained with Iba1 were imaged using a 20x objective, and all images were acquired with the same laser power and exposure settings. Images of neocortex from the corpus collosum to the rhinal fissure and whole hippocampus were acquired.

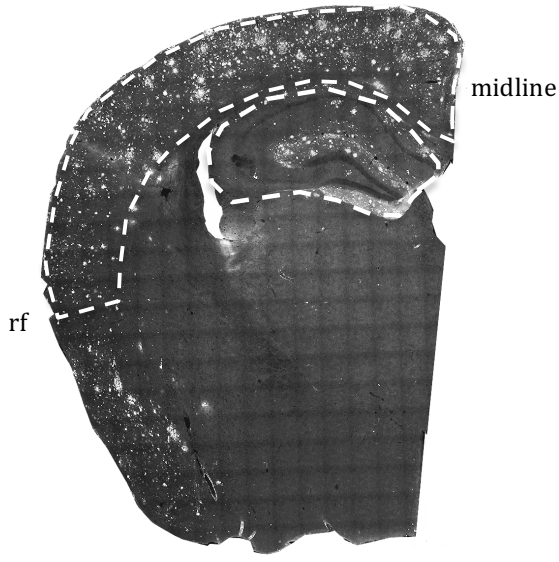

Figure 2.2. One hemisphere of coronal mouse brain section stained with the MOAB-2 antibody, outlining the regions of analysis for plaque load.

2.8 Random forest classification for the analysis of histology

Images of brain sections stained with Thioflavin-S, MOAB-2, Synaptophysin, and Iba1, were processed to 8-bit greyscale images, and segmented with a custom plugin for ImageJ. Segmentation included an automated random forest classifier (Sommer et al., 2011; Figure 2.3) which utilizes supervised machine learning. Supervised machine learning is an alternative method of analysing histological data to thresholding the images, and is more effective at discriminating image artefacts, is robust against variability in staining intensity, and is more objective relative to thresholding techniques (Holmes, Kapelner, & Lee, 2009; Sommer, Straehle, Kothe, & Hamprecht, 2011). Here, the classifier was trained with a random selection of example images for each marker, which were annotated in order to detect immunostaining from background pixels. A learning algorithm was developed taking into account a combination of image metrics that provide the most reliable discrimination of signal pixels from background (e.g. colour, edges, texture). The custom random forest classifier used in these studies, was developed by O'Mara and Kirkcaldie (Manuscript in preparation). It should be noted that images of cortex and hippocampus sections display a tiling-edge effect (e.g. Figure 5.2), and so values may not be absolute.

Figure 2.3. APP/PS1 mouse neocortex stained with Thioflavin-S and resulting image following random forest segmentation shows plaques for analysis.

2.8 Western blotting and ELISA

Neocortex and hippocampus were homogenized in RIPA buffer (Sigma) containing a protease (Roche diagnostics) and phosphatase inhibitor cocktail (AG Scientific). The samples were centrifuged at 13000 RPM for 15 minutes, rotated for a further 30 minutes, and centrifuged again at 4°C for 15 minutes at 13000 RPM. The resulting supernatant was removed and stored at -80°C for protein analysis. The protein concentrations of samples were determined using the Bradford assay.

2.8.1 Western blotting

For western blotting, samples were prepared as a total volume of 10µl containing 10µg of protein per lane. The samples were separated (3 repeats/sample) on a 12% NuPage Novex Bis-Tris gel (Invitrogen) by electrophoresis at 200V for 20 minutes at room temperature. Following, proteins were transferred to an activated

PVDF membrane at 20V for 60 minutes. Membranes were blocked for 2 hours in 5% commercial skim milk powder. Membranes were incubated in primary antibodies overnight at 4°C in combinations of rabbit anti-synaptophysin (1:2000, Millipore); mouse anti-VGlut1 (1:1000, Millipore); mouse anti-PSD-95 (1:1000, Abcam) mouse anti-GAD65; mouse anti-GAD67 (1:1000, Millipore), rabbit anti-Gephyrin (1:1000, Abcam) and anti-β-actin (1:5000, Sigma-Aldrich) (Table 2.1). Membranes were washed in Tris-buffered saline with 0.1% Tween-20 (Sigma) and incubated in species-appropriate secondary antibodies (1:7000, Dako, Table 2.2). Bands were visualised by staining with a chemiluminescent substrate kit (Millipore).

2.8.2 $A\beta_{42}$ ELISA

Neocortex and hippocampus were homogenized in RIPA buffer (Sigma) containing a protease (Roche diagnostics) and phosphatase inhibitor cocktail (AG Scientific). The samples were centrifuged at 13000 RPM for 15 minutes, rotated for a further 30 minutes, and centrifuged again at 4°C for 15 minutes at 13000 RPM. The resulting supernatant was removed and stored at -80°C for protein analysis. The protein concentrations of samples were determined using the Bradford assay. For the quantitation of human $A\beta_{42}$, a sandwich antibody ELISA was performed according to the manufacturer's instructions (KHB3441, Invitrogen). APP/PS1 mice (n = 5 per group) were terminally anaesthetized first with gas anaesthesia (isoflurane) followed by sodium pentobarbitone (100 mg/kg delivered intraperitoneally) and perfused transcardially with PBS (0.1M). Postmortem brains were removed and the cortex and hippocampus were dissected and immediately frozen in liquid nitrogen. Cortex and hippocampal samples were stored at -80°C for later analysis. Soluble human $A\beta_{42}$ levels were normalized to total protein levels and expressed as picogram of $A\beta_{42}$ content per milligram of total protein (pg/mg). Optical densities were read at 450 nm on a

microplate reader (SpectraMax, Molecular Devices), and concentrations of $A\beta_{42}$ were determined by comparison to the standard curve using a 4-parameter algorithm.

2.8.3 Corticosterone ELISA

In order to determine if there were differences in levels of stress between the housing conditions and genotypes, blood was collected at time of perfusion by cardiac puncture, performed at the start of the dark period (3 p.m.). Blood samples were centrifuged for 5 minutes at 13 000 RPM and the resulting sera was collected. The sera samples were diluted at 1:100 in the buffer provided, and levels of serum corticosterone were measured by a competitive commercial ELISA kit (ab108821; Abcam) according to manufacturer instructions. Optical densities were read at 450 nm on a microplate reader (SpectraMax, Molecular Devices), and concentrations of corticosterone were determined by comparison to the standard curve using a 4-parameter algorithm.

2.8.4 BDNF ELISA

Each sample was prepared in duplicate and diluted in coating buffer (1:100; 60% $NaHCO_3$, 30% $Na2CO_3$ in distilled water), and 50 µl of diluted sample was added per well to a 96-well flat-bottomed plate (Costar 5395, Sigma-Aldrich) and incubated at 4°C overnight. Following overnight incubation, the plate was washed five times with washing buffer (0.05% tween-20 in 0.01M PBS). Following, 100 µl of blocking buffer (5% fetal calf serum in 0.01M PBS) was added to each well and incubated at 37°C for 30 minutes. Following five washes, 50 µl of diluted primary BDNF antibody (1:500; Santa Cruz, Table 2.1) was added to each sample well, and incubated at room temperature for 1 hour. Five washes were undertaken and the secondary HRP antibody was added (1:2000; anti-Rabbit, Dako, Table 2.2) and incubated at room temperature for 45 minutes. Following washing, 100 µl of freshly prepared Tetramethylbenzidine

(TMB; Sigma-Aldrich) substrate was applied to each well for 10 minutes, and 0.1M Sulphuric acid was added to stop the colour reaction. Optical densities were read at 450 nm on a microplate reader (SpectraMax, Molecular Devices), and concentrations of BDNF were determined by comparison to the standard curve using a 4-parameter algorithm. Values were averaged between the duplicate samples, and expressed as a percentage relative to Wt controls.

2.9 Statistical analyses

Analyses were performed using IBM SPSS (Version 20;). Statistical analyses were performed using independent t-tests, two-way analysis of variance (ANOVA), and repeated measures ANOVA. A statistically significant two-way ANOVA was followed up by separate independent t-tests, where the variables considered were genotype (Wt or Tg) and housing condition (SH or EE). When the variables considered were SH, EE, and EE+, a statistically significant two-way ANOVA was followed up with *post hoc* tests with Bonferroni correction applied for multiple comparisons. Means were reported as $\pm$ the standard error of the mean (SEM). The magnitude of differences between the means were reported as Cohen's *d.* Values of $p < .05$ for differences between group means were classified as statistically significant.

Table 2.1. List of primary antibodies

Antibody name	*Host organism (Clone)*	*Isotype*	*Immunizing agent*	*Source (catalogue number)*	*Dilution*
MOAB-2	Mouse monoclonal	IgG2b	C-terminal for Aβ40 and Aβ42 (Youmans et al., 2012)	Novus Biologicals (NBP2-13075)	1:2000 (IHC)
Synaptophysin	Rabbit polyclonal	IgG	Synthetic peptide of human synaptophysin (313 amino acids) (Rehm, Wiedenmann, & Betz, 1986)	Millipore (AB9272)	1:200 (IHC) 1:2000 (WB)
VGlut1	Mouse monoclonal	IgG1	Recombinant protein from rat VGlut1 (560 amino acids) (Fazzari et al., 2014)	Millipore (MAB5502)	1:1000 (WB)
PSD-95	Mouse monoclonal (clone 6G6-1C9)	IgG2a	Recombinant protein from rat PSD-95 (Li et al., 2010)	Abcam (Ab2723)	1:1000 (WB)
Gephyrin	Rabbit polyclonal	IgG	Synthetic peptide of human Gephyrin (amino acids 396-445) (Harvey et al., 2004)	Abcam (Ab83401)	1:1000 (WB)
GAD65	Mouse monoclonal (clone GAD-6)	IgG2a	Purified GAD65 from rat (585 amino acids) (Besser et al., 2015)	Millipore (MAB351)	1:1000 (WB)
GAD67	Mouse monoclonal (clone 1G10.2)	IgG2a	Recombinant GAD67 protein (594 amino acids) (Fong, Stornetta, Foley, & Potts, 2005)	Millipore (MAB5406)	1:1000 (WB)
β-actin	Mouse monoclonal (clone AC-74)	IgG2a	Modified β-cytoplasmic actin N-terminal peptide (Arellano, Guadiana, Breunig, Rakic, & Sarkisian, 2012)	Sigma-Aldrich (A5316)	1:5000 (WB)
BDNF	Rabbit polyclonal	IgG	Epitope mapping, internal region of Human BDNF (amino acids 128-147) (Flores-Otero & Davis, 2011)	Santa Cruz (SC-546)	1:500 (ELISA)
Iba1	Rabbit		Synthetic peptide corresponding to C-terminus of Iba1from rabbit (Ito et al., 1998)	Wako (019-19741)	1:1000 (IHC)

Table 2.2. List of secondary antibodies

Emission (nm)	*Species/Reactivity*	*Dilution*	*Supplier (catalogue number)*
488	Goat/Mouse IgG1	1:1000	Molecular probes (A-11001)
546	Goat/Mouse IgG2b	1:1000	Molecular probes (A-21143)
488	Goat/Rabbit IgG	1:1000	Molecular probes (A-11008
546	Goat/Rabbit	1:1000	Molecular probes (A-11035)

Chapter 3

3. Mid-life environmental enrichment increases synaptic density in CA1 in a mouse model of Aβ-associated pathology and positively influences synaptic and cognitive health in healthy ageing

3.1 Introduction

Interventions to reduce the burden of dementia-causing diseases are urgently needed given global ageing and the current lack of effective therapeutic approaches. Epidemiological investigations suggest an active cognitive lifestyle may offer protection against the clinical expression of dementia (Valenzuela, Brayne, Sachdev, Wilcock, & Matthews, 2011), potentially by building resilience to the underlying pathology and compressing cognitive morbidity to later stages of disease. In order to mimic the benefits of enhanced cognitive stimulation, an EE paradigm in experimental animals is often employed. An EE paradigm typically involves the manipulation of an animals' environment in order to facilitate sensory, cognitive, and motor stimulation (van Praag et al., 2000; Nithianantharajah & Hannan, 2006).

There is a wealth of evidence supporting EE-induced cognitive and neural benefit in a range of animal models of neurodegenerative disease (Nithianantharajah & Hannan 2006). However, the reported effects on a hallmark pathological feature of AD, Aβ plaques, in familial Alzheimer's disease (FAD) animal models have been variable. Following an EE intervention, reduction (Lazarov et al., 2005; Costa et al., 2007; Herring et al., 2011), no change (Arendash et al., 2004; Wolf et al., 2006; Cotel, Jawhar, Christensen, Bayer, & Wirths, 2012), or an exacerbation of Aβ pathological burden (Jankowsky et al., 2003; 2005) has been reported in transgenic mice expressing human FAD-related gene mutations. Notably, many of these EE studies have involved such stimulation (EE) from weaning or in early-life. Despite the variation between

studies regarding Aβ pathological alterations following EE, there is a consensus of marked protection of cognitive function following this intervention.

In this regard, the underlying mechanism allowing for cognitive protection is elusive. EE in other models may influence synaptic connectivity in order to provide cognitive benefit (Nithianantharajah & Hannan, 2006). Moreover, BDNF, a protein expressed widely throughout the CNS, vital for the maintenance, survival, and growth of neurons (Mattson et al., 2004) has been implicated in both AD and EE. As BDNF mediates synaptic plasticity and cognitive function (e.g. Murer et al., 2001; Lu, 2003), it is thought that BDNF may be critically involved in the pathophysiology underlying cognitive decline in AD. Moreover, reduced levels of BDNF are found in the hippocampus and frontal and parietal cortices of the AD brain (Ferrer et al., 1999; Hock et al., 2000). BDNF promotes synapse formation (Park & Poo, 2013) and thus could be a potential target for diseases of synaptic plasticity failure, such as in AD. To date, BDNF has not been able to be delivered across the blood-brain barrier (Lu et al., 2013). However, some evidence suggests EE may be able to increase endogenous levels of BDNF in healthy animals (Ickes et al., 2000; Ramírez-Rodríguez et al., 2014; Novkovic et al., 2015).

With respect to human interventions of relevance to dementia and EE, the benefit of early-life cognitive stimulation, in the form of education, on later-life cognitive function has been well reported (e.g. Anstey & Christensen 2000; Lenehan, Summers, Saunders, Summers & Vickers, 2014). However, research on the potential protective effects of later-life cognitive engagement and enrichment is limited. The Tasmanian Healthy Brain Project (THBP; Summers et al., 2013) has produced the first research on the potential benefit of formal late-life education on cognitive reserve (Lenehan et al., 2015). However, investigation of later-life EE in animal models of

neuropathology has been limited. Moreover, whether cognitive intervention introduced at the inception of AD-neuropathology produces benefit is unknown. This concept is difficult to test in human cases considering dementia is typically detected many years after the AD-related neuropathology forms (Braak & Del Tredici, 2011).

In the current study, we investigated the effects of an EE intervention in a FAD transgenic model ($APP_{swe}PS1_{dE9}$ line; APP/PS1) from 6 months of age to model an intervention targeted to mid-life. The APP/PS1 mouse model begins to show Aβ plaque pathology by 6 months of age (Garcia-Alloza et al., 2006) and most closely resembles earlier stages of human AD in terms of synapse loss and neuritic pathology (Mitew, Kirkcaldie, Dickson & Vickers, 2013a, b). It was hypothesized that EE would produce positive cognitive effects associated with less vulnerability of, or increased levels of synaptic markers, rather than a reduction in Aβ neuropathology.

We examined region-specific alterations in synapses following EE. The dorsomedial region of the murine prefrontal cortex, frontal area 2 (Fr2), involved in complex cognitive functions (Uylings, Groenewegen, & Kolb, 2003) was selected. In addition, we examined the hippocampal subregions CA1 and CA3 due to their role in spatial learning and memory (Anderson et al., 2006; Kesner, 2007). These regions are not only potential targets of EE evidenced by increases in working memory and spatial learning and memory in rodent models exposed to EE, but are also highly susceptible to AD (Vickers et al., 2000; Patrylo & Williamson, 2007; Morrison & Baxter, 2014).

3.2 Materials and Methods

3.2.1 Animals and EE protocol

Male transgenic mice expressing chimeric mouse/human amyloid precursor protein (APP) and mutant human presenilin 1 (PS1) on C57BL/6 background [B6.Cg-Tg (APPswe, $PSEN1_{dE9}$) 85Dbo/J] (APP/PS1; Jankowsky et al., 2004) were used for the present study (described in Chapter 2). All animals lived in standard housing (SH) conditions comprising group housing of 4-5 mice per 30 x 30 x 14 cm cage, *ad Libitum* access to food and water, an igloo, one small wooden stick and one tissue. $APP_{swe}PSEN1_{dE9}$ (APP/PS1; $n = 27$) and littermate wildtype control (Wt; $n = 21$) mice were randomly assigned to SH or EE conditions at 6 months of age, for the following 6 months (EE paradigm outlined in Chapter 2).

3.2.2 Cognitive and behavioural assessment general protocol

Mice were handled daily and habituated to the test room for 7 days prior to testing to minimize the effects of stress due to handling at time of testing. All cognitive testing procedures occurred at the same stage of the light cycle. Black curtains surrounded the test equipment during testing, in order to prevent the mice becoming distracted, or from using extra-maze cues. Testing equipment was cleaned with 70% ethanol between trials in order to prevent odour cues. The experimenter was blind to the genotype of animals. Noise was minimized and lighting was kept constant throughout all testing. Exclusion criteria for behavioural testing were defined prior to testing, and adhered to. All testing was recorded for later analysis using a JVC digital camera mounted to the ceiling.

3.2.2.1 Y Maze spatial short-term memory

All mice underwent a 10-minute acquisition trial, where a randomly assigned arm of the Y maze was blocked (novel arm). The testing phase began after a 1-hour inter-trial interval, where all three arms of the maze were open for exploration for 5 minutes. Y maze testing was recorded for later analysis, where the percentage of time spent in the novel arm was calculated as a percentage of the total time in all three arms. Testing was performed immediately before being assigned to differential housing, in order to establish baseline performance, after 3 months of differential housing and finally after 6 months at end-point.

3.2.2.2 Barnes maze spatial learning & long-term memory

Testing on the Barnes maze was performed following 6 months of differential housing. Mice were initially habituated to the maze by being able to freely explore the maze for two 5-minute adaptation trials. Twenty-four hours after adaptation, a seven-day training period began of two trials per day. The trial ended when the mouse had climbed into the hidden escape, or after 5 minutes had elapsed. Fourteen days after the 7-day training period had elapsed, long-term memory was assessed with four trials. The latency to reach the escape box in each trial was recorded for analysis.

3.2.3 Tissue collection

Following the final day of behavioural testing, mice were terminally anaesthetized first with gas anaesthesia (isoflurane) followed by sodium pentobarbitone (100 mg/kg delivered intraperitoneally). For histology (Wt $n = 11$; APP/PS1 $n = 17$), animals were perfused transcardially with 4% paraformaldehyde (PFA) in 0.1M phosphate buffered saline (PBS pH 7.4). Postmortem brains were transferred to 18%

then 30% sucrose solutions overnight. Brains for histology were serially sectioned on a cryostat (Leica CM 1850) in 40µm coronal sections. Sections used for analysis were from bregma 2.0 - ¯3.0 mm according to the stereotaxic mouse brain atlas (Paxinos & Franklin, 2008). For Western blotting procedures, animals (Wt n = 10; APP/PS1 n = 10) were transcardially perfused with PBS (0.1M). Postmortem brains were removed and the neocortex and hippocampus were dissected and immediately frozen in liquid nitrogen, and samples were stored at -80°C for later analysis.

3.2.4 Antibody characterization

All antibodies used in the present study are commercially available and have been previously characterized (see Table 2.1 for references). Optimal antibody concentrations were determined for each antibody. Control experiments were performed by omitting primary antibodies, which eliminated all immunoreactivity. Aβ deposits were detected by immunohistochemistry using a mouse monoclonal antibody (MOAB-2) with an epitope at residues 1-4 (manufacturer information). The MOAB-2 antibody is raised against human Aβ, and recognises human unagreggated, oligomeric, and fibrillar forms of $A\beta_{42}$, but not APP (Youmans et al., 2012). Previous work from our lab has confirmed the specificity and labelling pattern of the MOAB-2 antibody, which co-labels with the commonly used anti-Aβ antibody, 6E10 (Collins et al., 2015). Synaptic puncta were visualized by immunolabelling with the pan-presynaptic marker, synaptophysin. The synaptophysin antibody is a polyclonal antibody raised in rabbit, the immunogen is a full-length (313 amino acids) synthetic peptide from human synaptophysin, and labels a single band at approximately 38 kDa on western blots (manufacturer's information).

The following antibodies were used for Western blot in the present study, and recognise the expected band on a Western blot of mouse brain tissue. The molecular sizes of the immunoreactive bands are: mouse anti-VGlut1, 60 kDa; mouse anti-PSD-95, 95 kDa; Rabbit anti-Gephyrin, 80 kDa; mouse anti-GAD65, 65 kDa; mouse GAD67, 67 kDa; mouse anti-β-actin (internal control), 42 kDa. The antibody rabbit anti-BDNF was used for ELISA, the antibody detects precursor and mature BDNF, and labels bands at 32 kDa (precursor) and 14 kDa (mature) (manufacturer information). Table 2.1 shows full antibody information.

3.2.5 Quantitation of Aβ plaques

For the quantitation of Aβ deposits, antigen retrieval by formic acid treatment was performed in order to enhance immunoreactivity for the visualization of Aβ deposits (Kai et al., 2012). Ten sections evenly spaced across the rostrocaudal extent of the brain (bregma 2.0 - ¯3.0 mm) were incubated in formic acid (Sigma-Aldrich) at room temperature for 8 minutes, followed by 6 x 10 minute PBS washes. Free-floating sections were washed for 3 x 10 minutes in 0.25% Triton-X-100 and incubated with serum-free protein block (Dako) for 15 minutes at room temperature. The sections were immunolabelled with the MOAB-2 antibody (1:2000; Novus Biologicals, Table 2.1) which specifically labels mouse and human un-aggregated Aβ, following the protocol outlined in Collins, King, Woodhouse, Kirkcaldie and Vickers (2015). MOAB-2 labelling was visualized by incubation in Alexa-fluorophore conjugated secondary antibody (1:1000; Molecular Probes, goat anti-mouse IgG2b-546, Table 2.2) and cover-slipped using Dako fluorescent mounting medium.

3.2.6 Quantitation of synaptophysin immunoreactive puncta

To determine region-specific alterations in synaptic density, synaptophysin immunoreactive puncta were quantitated histologically in the dorsomedial region of the murine prefrontal cortex, frontal area 2 (Fr2; Van De Werd, Rajkowska, Evers, & Uylings, 2010) and the hippocampal subregions CA1 and CA3 (Paxinos & Franklin, 2008). Three sections containing Fr2 (bregma 1.98 – 0.38 mm) and three sections that contained CA1 and CA3 (bregma ¯1.28 - ¯2.12 mm) were immunostained with primary anti-synaptophysin antibody (1:200; Millipore, Table 2.1) according to standard procedures (Collins et al., 2015) using Alexa-fluorophore secondary antibody, goat-anti-rabbit IgG 594 (Molecular Probes, Table 2.2). To highlight architecture, sections were also incubated with the nuclear stain DAPI (5 μg/mL; Invitrogen) for 5 minutes at room temperature. Sections were mounted using fluorescent mounting medium (Dako).

3.2.7 Image acquisition

In order to determine Aβ plaque load in the neocortex and hippocampus, images were obtained with a Leica DM fluorescence microscope on a 10x objective and NIS Elements imaging software. Ten sections evenly spaced from the rostral to caudal extent of the neocortex from bregma position 2.0 to -3.0 mm were imaged for MOAB-2 Aβ plaque load. The left side of the neocortex was imaged from the midline to the rhinal fissure. Images of the whole hippocampus were taken between bregma position -1.22 and -2.46 mm of 3-5 sections per animal. Aβ plaque load (defined as percentage area occupied by MOAB-2 immunoreactivity) was determined by applying a custom plugin for ImageJ to the plaque images, which automatically segmented images as plaques or background pixels by random forest classification, as described by Sommer et al. (2011). The classifier was trained with a random selection of example plaque

images from the data set, which were annotated in order to distinguish plaques from background pixels.

Imaging of synaptophysin labelling was performed on a Perkin-Elmer Ultraview VOX confocal imaging system with Volocity 6.3 imaging software. All images were acquired with the same laser power and exposure settings, using a 60x objective. Five images (image window size = 118.15 x 118.15μm)/section were taken within Fr2, CA1, and CA3 subregions across three sections/animal. For APP/PS1 animals, Aβ plaque free regions were included in the analysis. These images then underwent image segmentation and random forest classification in order to distinguish synaptic puncta from background pixels using the custom imageJ plugin as described for plaque load analysis. An imageJ watershed algorithm with Gaussian blurring ($\sigma = 1$) was applied to the segmented images. Particles ranging from 0.15 to 2.0μm^2 were quantified (Mitew et al., 2013a). Synaptic density was calculated as the number of synaptophysin immunolabelled puncta per field (118.15 x 118.15μm), corrected for cell body area.

3.2.8 Western blotting

The right side of the neocortex and hippocampus were homogenized in RIPA buffer (Sigma) containing a protease (Roche diagnostics) and phosphatase inhibitor cocktail (AG Scientific). The samples were centrifuged at 13000 RPM for 15 minutes, rotated for a further 30 minutes, and centrifuged again at 4°C for 15 minutes at 13000 RPM. The resulting supernatant was removed and stored at -80°C for protein analysis. The protein concentrations of samples were determined using the Bradford assay. Samples were prepared as a total volume of 10μl containing 10μg of protein per lane. The samples were separated (3 repeats/sample) on a 12% NuPage Novex Bis-Tris gel (Invitrogen) by electrophoresis at 200V for 20 minutes at room temperature. Following, proteins were transferred to an activated PVDF membrane at 20V for 60 minutes.

Membranes were blocked for 2 hours in 5% commercial skim milk powder. Membranes were incubated in primary antibodies overnight at 4°C in combinations of rabbit anti-synaptophysin (1:2000, Millipore); mouse anti-VGlut1 (1:1000, Millipore); mouse anti-PSD-95 (1:1000, Abcam) mouse anti-GAD65; mouse anti-GAD67 (1:1000, Millipore), rabbit anti-Gephyrin (1:1000, Abcam) and anti-β-actin (1:5000, Sigma-Aldrich) (Table 2.1). Membranes were washed in Tris-buffered saline with 0.1% Tween-20 (Sigma) and incubated in species-appropriate secondary antibodies (1:7000, Dako, Table 2.2). Bands were visualised by staining with a chemiluminescent substrate kit (Millipore).

3.2.9 BDNF Enzyme-linked immunosorbent assay (ELISA)

Neocortex and hippocampal samples were prepared as described for Western blotting. Briefly, each sample was prepared in duplicate and diluted in coating buffer (1:100; 60% $NaHCO_3$, 30% $Na2CO_3$ in distilled water), and 50 µl of diluted sample was added per well to a 96-well flat-bottomed plate (Costar 5395, Sigma-Aldrich) and incubated at 4°C overnight. Following overnight incubation, the plate was washed five times with washing buffer (0.05% tween-20 in 0.01M PBS). Following, 100 µl of blocking buffer (5% fetal calf serum in 0.01M PBS) was added to each well and incubated at 37°C for 30 minutes. Following five washes, 50µl of diluted primary BDNF antibody (1:500; Santa Cruz, Table 2.1) was added to each sample well, and incubated at room temperature for 1 hour. Five washes were undertaken and the secondary HRP antibody was added (1:2000; anti-Rabbit, Dako, Table 2.2) and incubated at room temperature for 45 minutes. Following washing, 100µl of freshly prepared Tetramethylbenzidine (TMB; Sigma-Aldrich) substrate was applied to each well for 10 minutes, and 0.1M Sulphuric acid was added to stop the colour reaction. Optical densities were read at 450 nm on a microplate reader (SpectraMax, Molecular

Devices), and concentrations of BDNF were determined by comparison to the standard curve using a 4-parameter algorithm. Values were averaged between the duplicate samples, and expressed as a percentage relative to Wt controls.

3.2. Statistical analysis

Analyses were performed using IBM SPSS (Version 20). Statistical analyses were performed using independent t-tests, two-way ANOVA, and repeated measures ANOVA. A statistically significant two-way ANOVA was followed up by separate independent t-tests. Variables considered were genotype (Wt or Tg) and housing condition (SH or EE). Values of $p < .05$ for differences between group means were classified as statistically significant.

3.3 Results

3.3.1 Body weight

No significant differences in body weight were detected between groups, $F(1, 42) = 1.60$, $p = .21$.

3.3.2 Pre-intervention and follow-up short-term memory

In order to assess cognitive changes following exposure to EE, we first established baseline short-term memory (STM) function using the Y maze at six-months of age. An independent t-test revealed APP/PS1 animals spent significantly less time in the novel arm of the maze compared to the Wts ($t_{(46)} = 3.15$, $p = .003$), an indication of a STM deficit. Following 3 months of differential housing, a two-way ANOVA demonstrated no significant genotype x housing effect on Y maze performance, $F_{(1, 42)} = 2.26$, $p = .14$. However, when Wt and APP/PS1 groups were

analysed separately, an independent t-test demonstrated for APP/PS1 mice, those in EE spent significantly more time in the novel arm of the maze than those in SH ($t_{(18)}$ = 3.75, p = 0.002) (Figure 3.1A) (Table 3.1).

3.3.3 Post-intervention short-term memory

Following 6 months of differential housing, at 12 months of age, no significant genotype x housing effect on Y maze performance was detected by two-way ANOVA ($F_{(1, 43)}$ = 0.20, p = 0.66). Moreover, a two-way repeated measures ANOVA of the three testing time-points revealed that Y maze performance over time was not significantly affected by housing x genotype ($F_{(2, 82)}$ = 2.07, p = 0.13) (Figure 3.1A) (Table 3.1).

Table 3.1. Mean percentage time in novel arm of the Y maze

	SH Wt			**EE Wt**			**SH Tg**			**EE Tg**		
	M	(SD)	*n*	**M**	(SD)	*n*	**M**	(SD)	*n*	**M**	(SD)	*n*
Baseline 6 months	51.63	12.00	13	53.33	9.89	14	41.39	15.79	11	41.15	13.56	10
9 months	36.72	14.80	12	42.89	14.84	14	27.72	11.24	10	45.41	9.04	9
12 months	41.56	15.59	12	50.83	14.70	14	51.51	18.15	10	53.09	20.69	9

3.3.4 Post-intervention learning & long-term memory

A Greenhouse-Geisser corrected repeated measures ANOVA of learning on the Barnes maze demonstrated no significant main effect of genotype x housing on learning ($F_{(9.19, 280.18)}$ = 0.56, p = 0.87) (Figure 3.1B) (Table 3.2). In addition, a two-way ANOVA demonstrated no significant genotype x housing effect on long-term memory

performance ($F_{(1, 46)} = 2.78$, $p = 0.10$) (Figure 3.1C). A separate independent t-test of Wt animals, demonstrated that those in EE when compared to those in SH, demonstrated a significantly reduced latency to reach the escape on the LTM trial ($t_{(25)} = 2.07$, $p = 0.049$). However, analysis of the Tg animals revealed EE did not have a significant effect on LTM ($t_{(10.94)} = 0.39$, $p = 0.71$).

Table 3.2. Latency (s) to reach escape on the Barnes Maze learning trials

Trial	SH Wt M (SD)	EE Wt M (SD)	SH Tg M (SD)	EE Tg M (SD)
1	179.42 (81.67)	121.75 (86.16)	133.85 (78.96)	147.55 (78.21)
2	125.96 (65.14)	97.54 (62.53)	171.85 (99.52)	127.60 (99.85)
3	114.67 (86.33)	53.96 (44.96)	104.40 (46.80)	92.35 (80.99)
4	109.88 (70.04)	104.38 (90.87)	121.15 (75.10)	116.65 (92.72)
5	106.00 (73.37)	74.96 (83.07)	109.10 (78.01)	120.25 (87.36)
6	118.71 (87.78)	60.46 (82.12)	87.55 (43.27)	84.40 (49.37)
7	116.38 (92.00)	67.58 (48.01)	94.80 (73.18)	110.65 (58.37)

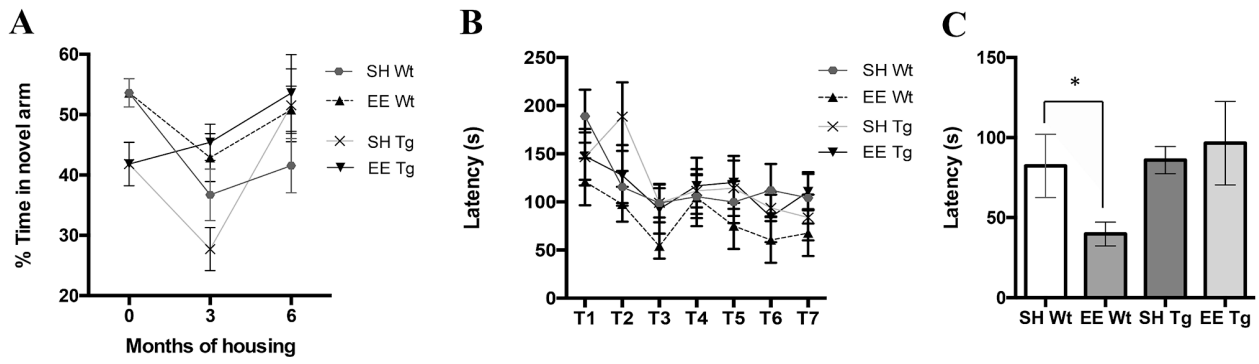

Figure 3.1. Y maze and Barnes maze performance. **A** Mean percentage of time spent in the novel arm of the Y maze (± SEM) at baseline, and after 3 and 6 months of differential housing. Baseline testing at 6 months of age demonstrated APP/PS1 mice spent significantly less time in the novel arm of the Y maze, $t_{(46)} = 3.15$, $p = 0.003$. After 3 months of housing, no genotype x housing effect on Y maze performance was detected, $F_{(1, 42)} = 2.26$, $p = 0.14$. Following 6 months of differential housing, no significant genotype x housing effect on Y maze performance was detected, $F_{(1, 43)} = 0.20$, $p = 0.66$. **B** Mean latency (± SEM) to reach the escape on the Barnes maze over 7 days of learning trials at 12 months of age. No significant main effect of genotype x housing on learning was detected, $F_{(9.19, 280.18)} = 0.56$, $p = 0.87$. **C** Long-term memory performance on the Barnes maze expressed as the mean latency (± SEM) to reach the escape over four trials. The EE Wt animals on average had significantly reduced latency to reach the escape, $t_{(25)} = 2.07$, $p = 0.049$. * $p < .05$, ** $p < .01$, *** $p < .001$.

3.3.5 Aβ plaque load

In order to determine whether EE introduced after the onset of Aβ neuropathology alters β-Amyloid load, we analysed MOAB-2 immunolabelling, an antibody that recognises human and mouse unaggregated, oligomeric, and fibrillar forms of Aβ42 and unaggregated Aβ40 (Youmans et al., 2012). An independent t-test of Aβ plaque load in the neocortex measured by MOAB-2 immunolabelling, did not differ significantly according to housing condition at 12 months ($t_{(15)} = 0.65$, $p = 0.53$) (Table 3.3). Additionally, no significant difference in hippocampal MOAB-2 immunolabelling was detected by independent t-test ($t_{(15)} = 0.18$, $p = 0.86$) (Table 3.3) (Figure 3.2).

Table 3.3. MOAB-2 load (% area) of Neocortex and Hippocampus

	SH $n = 9$ *M* (SEM)	EE $n = 8$ *M* (SEM)
Neocortex	29.44 (2.75)	25.76 (5.15)
Hippocampus	25.42 (2.35)	24.74 (2.92)

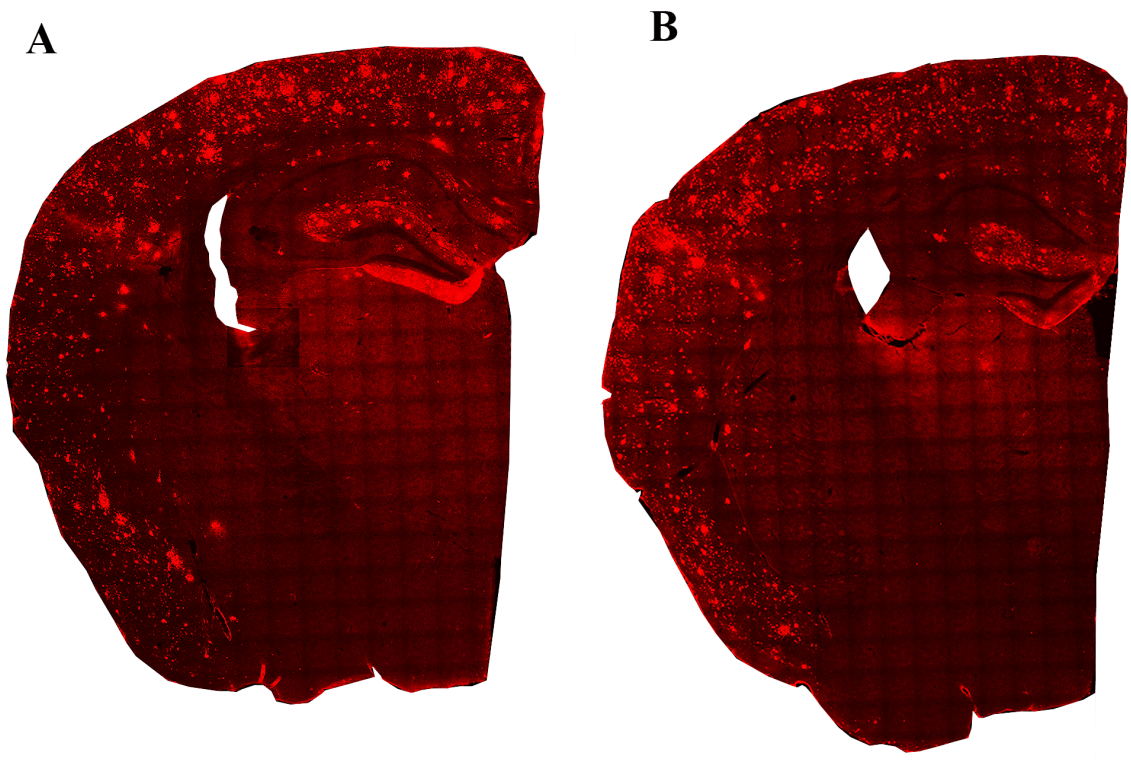

Figure 3.2. Representative APP/PS1 mouse brain sections stained with the MOAB-2 antibody. **A** APP/PS1 mouse brain coronal section with MOAB-2 positive plaques from SH. **B** APP/PS1 mouse brain coronal section with MOAB-2 positive plaques from EE.

3.3.6 Western blots of synaptic markers

In order to assess the effect of EE on synapses in APP/PS1 and Wt mice, we quantified levels of synaptic proteins in the neocortex and hippocampus by Western blotting, and analysed group differences by two-way ANOVA. A significant housing x genotype effect was detected for labelling with the pan-presynaptic protein, synaptophysin, in the neocortex ($F_{(1, 16)} = 8.12$, $p = 0.01$) (Figure 3.3B), and in the hippocampus ($F_{(1, 16)} = 9.43$, $p = 0.008$) (Figure 3.4B). Subsequent independent t-tests showed that Wt animals in EE, as compared to Wts in SH, had relatively increased

cortical ($t_{(8)} = 3.22$, $p = 0.01$) and hippocampal ($t_{(8)} = 3.51$, $p = 0.008$) synaptophysin levels.

To further examine whether these effects could be attributed to excitatory or inhibitory synaptic changes, antibody markers VGlut-1, PSD-95, gephyrin, and GAD65/67 were examined. No housing x genotype effect was detected in the excitatory synaptic marker VGlut-1 in the cortex ($F_{(1, 16)} = 0.15$, $p = 0.70$) (Figure 3.3C), or in the hippocampus ($F_{(1, 16)} = 0.007$, $p = 0.94$) (Figure 3.4C). No significant housing x genotype effect was detected for levels of the excitatory post-synaptic marker, PSD-95 in the cortex ($F_{(1, 16)} = 0.003$, $p = 0.95$) (Figure 3.3D). However, there was differential expression in the hippocampus ($F_{(1, 16)} = 8.99$, $p = 0.009$). When genotypes were analysed separately, Wt EE animals had higher hippocampal levels of PSD-95 compared to the Wts in SH ($t_{(8)} = 2.84$, $p = 0.02$) (Figure 3.4D). Gephyrin labelling was not significantly altered by the effect of housing x genotype in neocortex ($F_{(1, 16)} = 1.42$, $p = 0.25$) (Figure 3.3E) or in hippocampus ($F_{(1, 16)} = 1.13$, $p = 0.31$) (Figure 3.4E). However, analysing the Wt and APP/PS1 animals separately demonstrated that enriched Wt animals had significantly lower hippocampal gephyrin labelling as compared to those in SH ($t_{(8)} = 3.06$, $p = 0.02$). The inhibitory synaptic marker GAD65/57 was significantly affected by housing x genotype ($F_{(1, 16)} = 5.54$, $p = 0.03$). Analysing genotypes separately demonstrated increased GAD65/57 protein levels in neocortex of Wt EE animals ($t_{(7.45)} = 2.64$, $p = 0.03$) (Figure 3.3F). However, this effect was not detected in the hippocampus ($F_{(1, 16)} = 0.02$, $p = 0.90$) (Figure 3.4F).

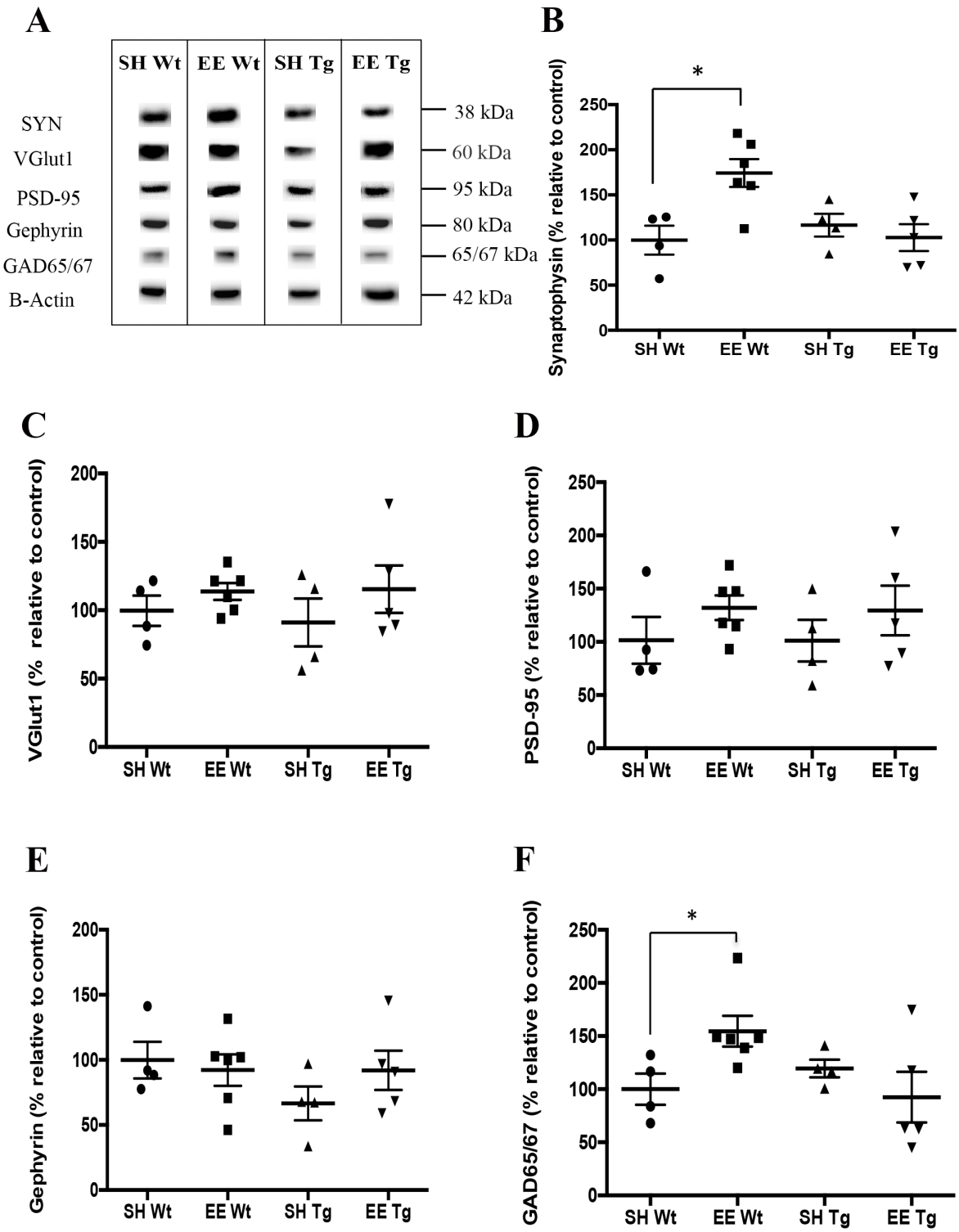
A
SH Wt
EE Wt
SH Tg
EE Tg
SYN
VGlut1
PSD-95
Gephyrin
GAD65/67
B-Actin
38 kDa
60 kDa
95 kDa
80 kDa
65/67 kDa
42 kDa
B
Synaptophysin (% relative to control)
*
C
VGlut1 (% relative to control)
D
PSD-95 (% relative to control)
E
Gephyrin (% relative to control)
F
GAD65/67 (% relative to control)
*

Figure 3.3 Mean synaptic protein levels expressed as a percentage relative to Wt control (± SEM) from neocortex samples. **A** Representative Western blot bands per group for each synaptic marker. **B** Synaptophysin: EE Wt mice had higher relative levels of cortical synaptophysin, $t_{(8)} = 3.22$, $p = 0.01$. **C** VGlut-1: No significant differences across housing x genotype were detected, $F_{(1, 16)} = 0.15$, $p = 0.70$. **D** PSD-95: No significant differences across housing x genotype were detected, $F_{(1, 16)} = 0.003$, $p = .95$. **E** Gephyrin: No significant differences across housing x genotype were detected, $F_{(1, 16)} = 1.42$, $p = 0.25$. **F** GAD65/67: Wt EE mice showed significantly increased levels of GAD65/67 compared to those in SH, $t_{(7.45)} = 2.64$, $p = 0.03$. * $p < .05$, ** $p < .01$, *** $p < .001$.

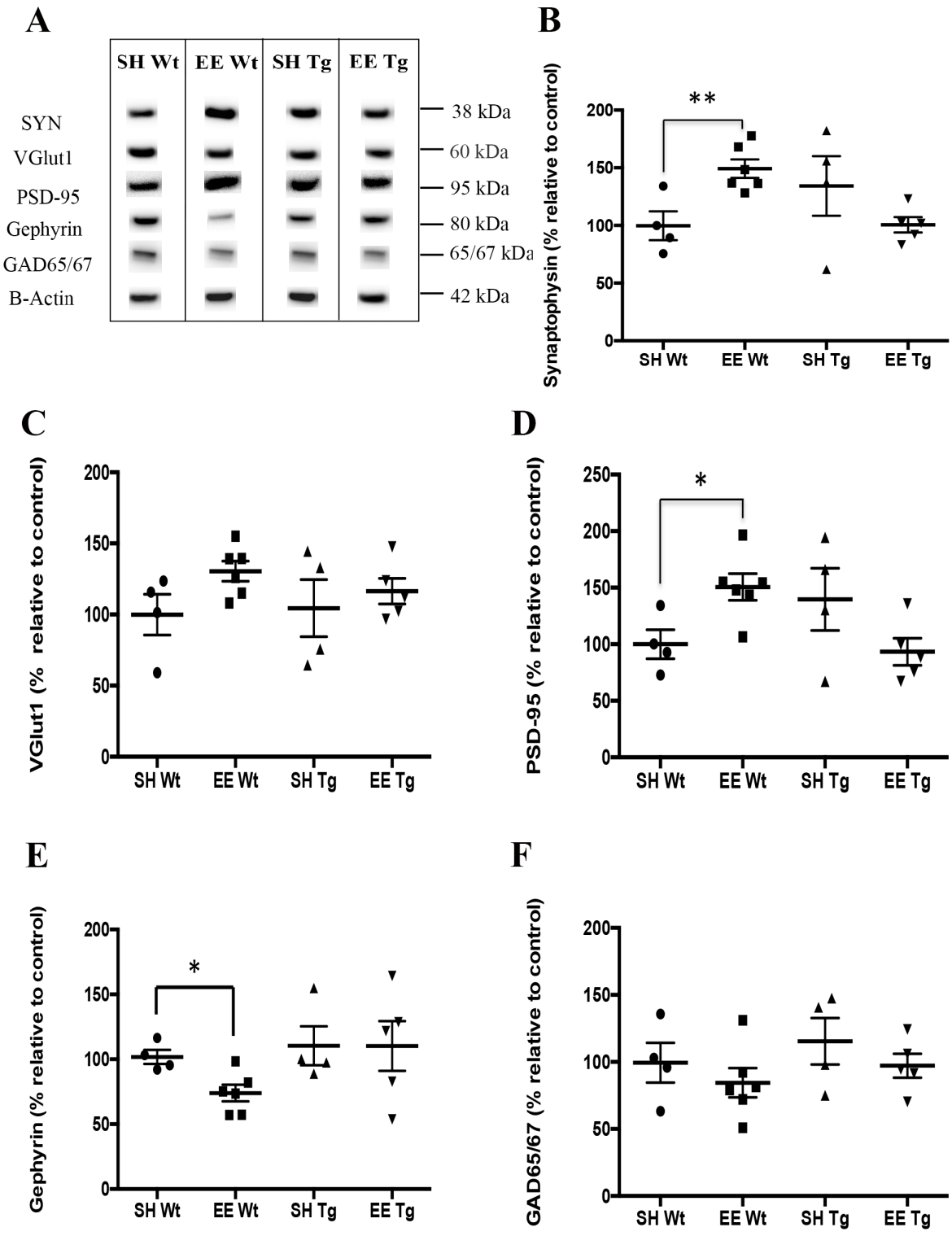
A
SH Wt
EE Wt
SH Tg
EE Tg
SYN
VGlut1
PSD-95
Gephyrin
GAD65/67
B-Actin
38 kDa
60 kDa
95 kDa
80 kDa
65/67 kDa
42 kDa
B
Synaptophysin (% relative to control)
**
C
VGlut1 (% relative to control)
D
PSD-95 (% relative to control)
*
E
Gephyrin (% relative to control)
*
F
GAD65/67 (% relative to control)
SH Wt
EE Wt
SH Tg
EE Tg

Figure 3.4. Mean synaptic protein levels expressed as a percentage relative to Wt control (± SEM) from hippocampus samples. **A** Representative Western blot bands per group for each synaptic marker. **B** Synaptophysin: EE Wt mice had significantly higher hippocampal synaptophysin compared to SH Wt mice, $t_{(8)} = 3.51$, $p = 0.008$. **C** VGlut-1: There was no significant housing x genotype effect on VGlut-1 expression in the hippocampus, $F_{(1, 16)} = 0.007$, $p = 0.94$. **D** PSD-95: Wt EE mice had significantly higher levels of PSD-95 compared to SH Wt $t_{(8)} = 2.84$, $p = 0.02$. **E** Gephyrin: EE Wt mice had significantly lower levels of hippocampal gephyrin as compared to SH Wt, $t_{(8)} = 3.06$, $p = 0.02$. **F** GAD65/67: No significant differences were detected across housing x genotype in the hippocampus, $F_{(1, 16)} = 0.02$, $p = 0.90$. * $p < .05$, ** $p < .01$, *** $p < .001$.

3.3.7 Synaptophysin immunolabelling in Fr2, CA1, and CA3

Quantification of immunolabelled synaptophysin puncta of the dorsomedial region of the prefrontal cortex, Fr2, showed no significant housing x genotype alterations in synaptophysin ($F_{(1, 24)} = 0.07$, $p = 0.79$) (Figure 3.5). Housing x genotype did not significantly influence CA1 synaptophysin immunolabelling ($F_{(1, 24)} = 0.94$, $p = 0.34$) (Figure 3.6). However, an independent t-test demonstrated that, for the APP/PS1 animals, EE produced a significant increase in synaptophysin density in CA1 ($t_{(16)} = 2.54$, $p = 0.02$). However, no significant effect of housing x genotype on levels of synaptophysin density was found within CA3, ($F_{(1, 24)} = 2.79$, $p = 0.12$) (Figure 3.7).

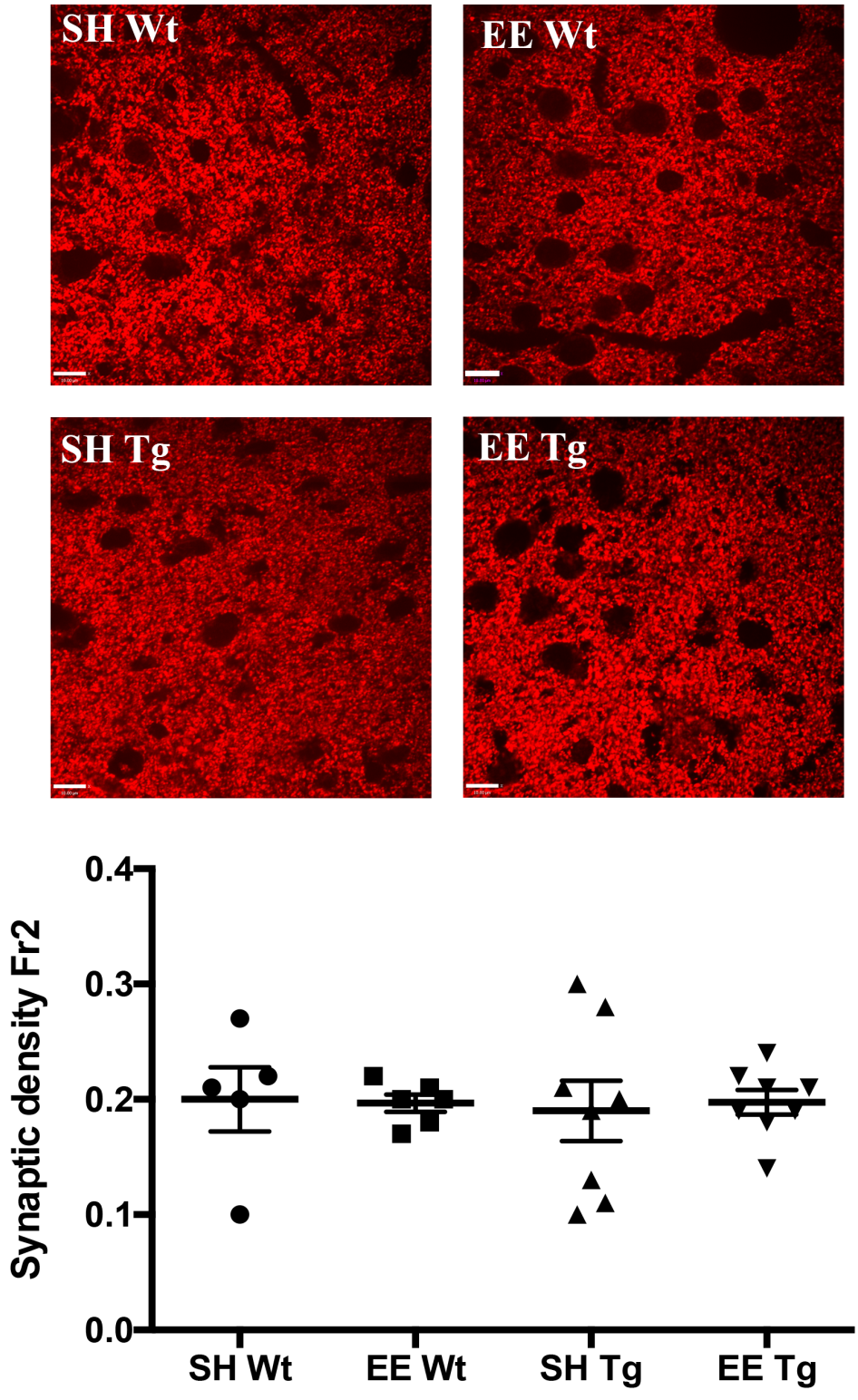

Figure 3.5. Synapse density in Fr2 of neocortex. Representative 60x (118.15 x 118.15 µm) synaptophysin staining in Fr2 per group, Scale bar = 20µm. Mean synaptophysin density (± SEM) of Fr2 showed no significant housing x genotype effect, $F_{(1, 24)} = 0.07$, $p = 0.79$.

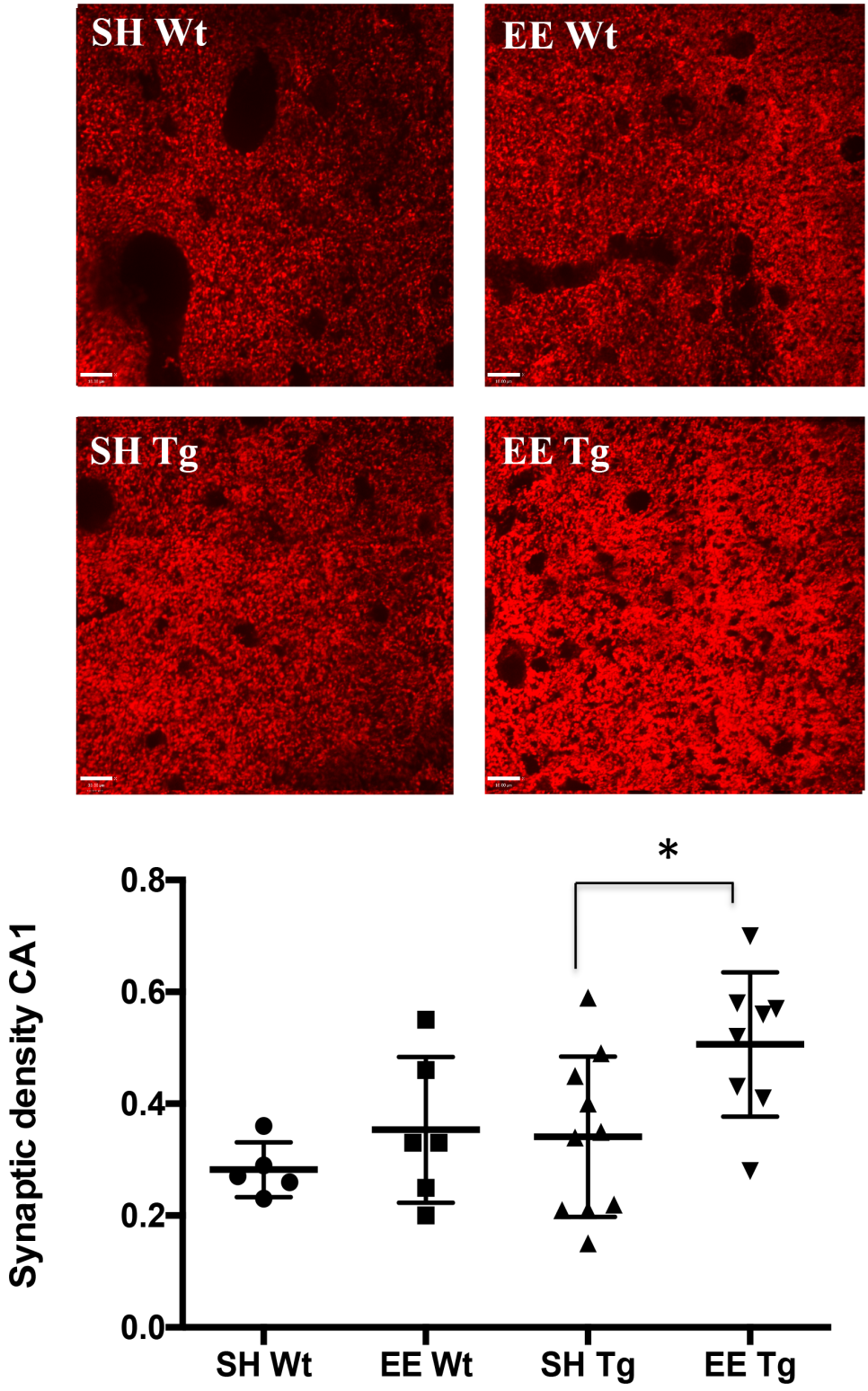

Figure 3.6. Synapse density in hippocampal subregion CA1. Representative 60x (118.15 x 118.15 µm) synaptophysin staining in CA1, Scale bar = 20µm. Mean synaptophysin density of CA1 (± SEM), the APP/PS1 mice from EE had significantly higher synaptophysin density in CA1, $t_{(16)} = 2.54$, $p = 0.02$, relative to the other groups. * $p < .05$, ** $p < .01$, *** $p < .001$.

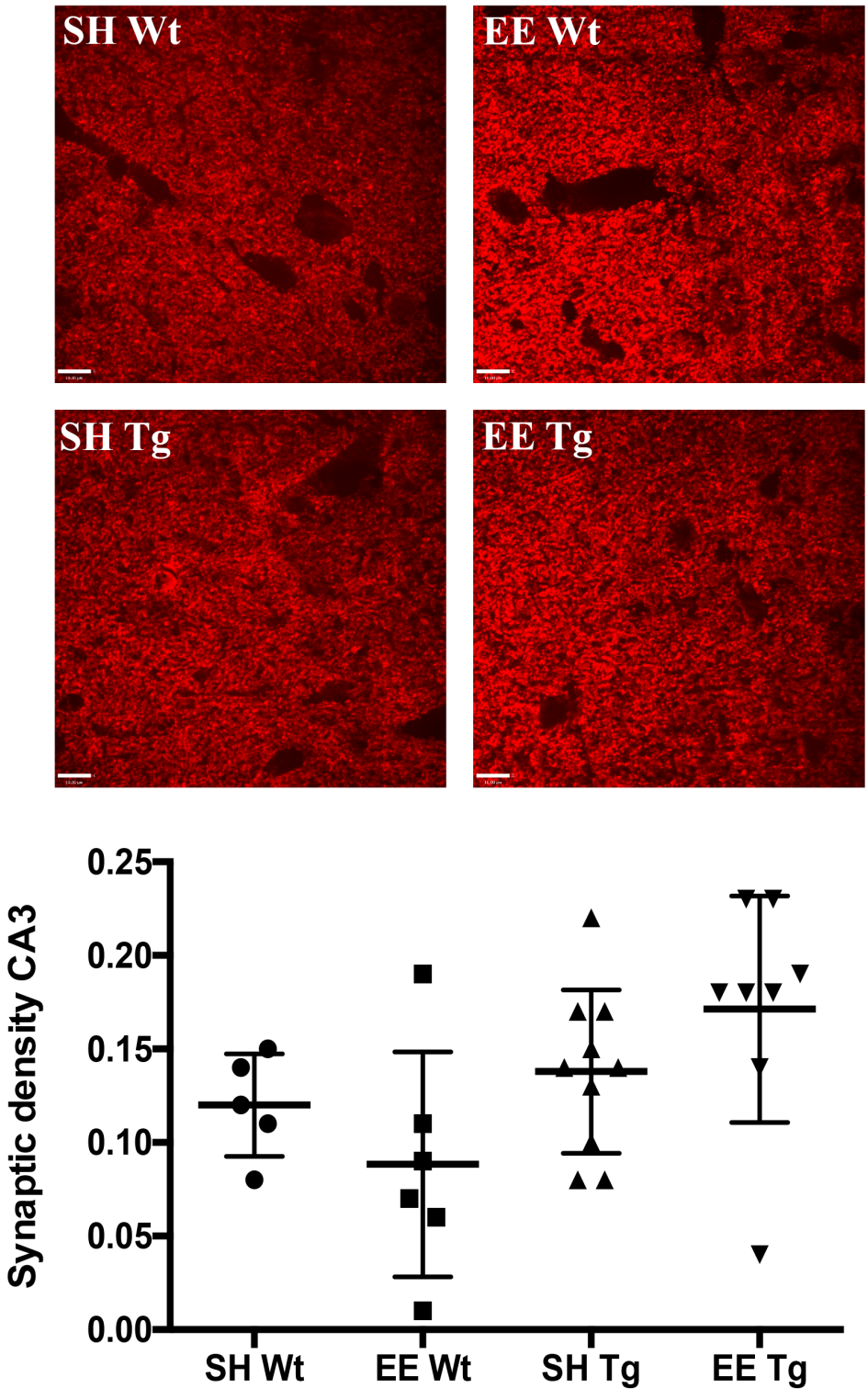

Figure 3.7. Synapse density in hippocampal subregion CA3. Representative 60x (118.15 x 118.15 µm) synaptophysin staining in CA3, Scale bar = 20µm. Mean synaptophysin density CA3 (± SEM) showed no significant housing x genotype effect, $F_{(1, 24)} = 2.79$, $p = 0.12$.

3.3.8 BDNF protein levels in neocortex and hippocampus

A two-way ANOVA demonstrated relative levels of BDNF were not altered in neocortex across genotype or housing condition ($F_{(2, 23)}$=0.88, p = 0.43) (Figure 3.8A). A two-way ANOVA also revealed no genotype x housing effect on hippocampal BDNF ($F_{(2, 23)}$ = 1.04, p = 0.37). However, when genotypes were considered separately, an independent t-test demonstrated a significant increase in hippocampal BDNF in APP/PS1 mice housed in EE compared to mice housed in SH ($t_{(8)}$ = 3.39, p = 0.009) (Figure 3.8B).

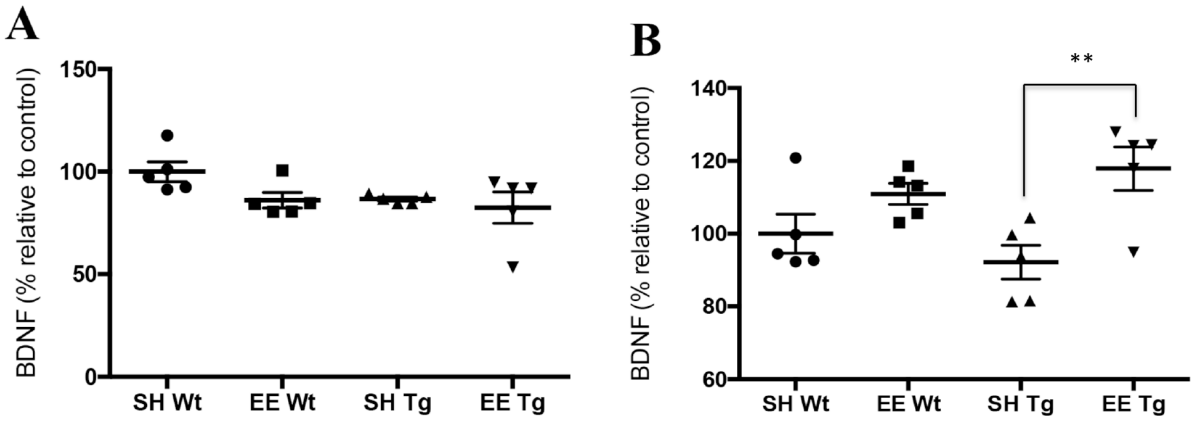

Figure 3.8. BDNF protein levels relative to Wt control (% expression ± SEM). **A** BDNF expression in neocortex is not altered by genotype or housing, $F_{(2, 23)}$=0.88, p = 0.43. **B** BDNF expression in hippocampus was elevated in APP/PS1 mice housed in EE relative to the SH condition, $t_{(8)}$ = 3.39, p = 0.009. * p <.05, **p <.01, ***p <.001.

3.4 Discussion

The global scale related to the predicted rise in the prevalence of AD (Prince et al., 2015) has led to increased research strategies aimed at delaying the onset of dementia. A substantial literature supports the proposal that a stimulating lifestyle beginning in early-life has beneficial later-life effects on cognitive ageing, potentially by buffering against pathological damage, compressing cognitive morbidity to later years. However, the effect of a cognitive intervention targeted at mid-life is not well understood. Moreover, whether cognitive stimulation can enhance the compensatory capacity of synaptic connectivity for existing pathology is unknown. Here, the effect of mid-life EE in experimental models of normal ageing and Aβ neuropathology was investigated.

The results of the present study demonstrate that EE initiated after Aβ plaque deposition has begun does not modify the course of subsequent amyloid pathology. However, EE differentially produced changes in cognitive function and synaptic markers. The data demonstrated the relationship between EE and synaptic health and cognitive function was not straightforward. Rather, it was found that EE in healthy ageing mice led to increased inhibitory synaptic markers in the cortex and elevated excitatory receptor markers in the hippocampus, along with superior long-term memory function. However, relative to Wt mice, and perhaps due to increased mutant APP/PS1 expression or AD-related pathology, the APP/PS1 mice did not show as extensive changes in synaptic protein levels or in cognitive function afforded by EE. However, EE produced an increase in synaptic density in CA1 in this model of Aβ neuropathology, and ameliorated some aspects of cognitive dysfunction associated with ageing and the APP/PS1 genotype.

With regards to the current study, there have been conflicting reports regarding the age of cognitive impairment onset in the APP/PS1 mouse model of AD. In the current report, a STM deficit was observed in APP/PS1 mice at 6 months of age. Other reports support the finding of the current study, where a STM deficit was observed at 6 months of age (Zhang et al., 2006; Aso et al., 2012; Izco et al., 2014). One explanation for an early STM deficit might be the dramatic increase in Aβ deposition at this time-point (Izco et al., 2014), resulting in neural disruption at the microcellular level.

Y maze testing after 3 months of EE demonstrated improved STM performance by the APP/PS1 animals. This finding suggested EE can abate the initial STM deficit in this mouse line. However, this beneficial effect did not persist at 12 months of age, with STM performance being similar across all animal groups. Moreover, Barnes maze testing at 12 months demonstrated superior LTM following 6 months of EE in Wt mice. This finding suggests that LTM function in ageing can benefit from EE, however, the presence of Aβ pathology may diminish this benefit. Although the present study had a relatively large sample size for FAD transgenic studies (Table 1), this enrichment paradigm may have induced more subtle cognitive changes that we did not have power to detect in the current study. One other interpretation for the lack of cognitive benefit to the APP/PS1 mice following 6 months of stimulation, is the increasing burden of Aβ pathology by 12 months attenuated the effect of EE. The results must also be interpreted with the features of this mouse model in mind, this APP/PS1 model being most closely aligned to the long, preclinical stage of human AD (Mitew et al. 2013a, b).

Consistent with several studies, the current data showed that Aβ plaque burden was not modified by EE (Arendash et al., 2004; Jankowsky et al., 2003; 2005; Wolf et al., 2006; Cotel et al., 2012), indicating that this intervention does not modify disease process progression. However, other studies have reported EE to directly attenuate Aβ

neuropathology (Lazarov et al., 2005; Cracchiolo et al., 2007). Potential discrepancies may be due to the time-point at which enrichment was initiated. The majority of studies have investigated EE introduced at weaning, or in early-life, and before the deposition of Aβ plaque pathology. Arendash et al. (2004) in an APP_{SW} model of AD, also demonstrated that later-life EE did not reduce Aβ load. Relatedly, Verret et al. (2013) reported decreased Aβ load following early-life EE, whereas EE introduced later in life did not lead to reductions in Aβ burden. These findings together suggest that EE does not have a modifying effect on plaque formation once Aβ deposition has commenced.

EE as a potential non-pharmacological intervention may have effects on the structural plasticity of synapses. In this respect, there have been a number of studies that have demonstrated synaptic changes as a result of EE (Nithianantharajah & Hannan, 2006). An increase in the expression of synaptophysin has been demonstrated following EE in healthy Wt animals in the neocortex and hippocampus (Nithianantharajah et al., 2004; Lambert, Fernandez, & Frick, 2005; Birch, Mcgarry, & Kelly, 2013). However, these results were obtained in young animals, and the relationship between enrichment, synaptophysin, and cognitive function in ageing is less clear. The present study demonstrated EE in ageing promotes synaptophysin expression in cortical and hippocampal regions, and is associated with relatively superior LTM performance. It has been suggested that, as synaptophysin is a component of neurotransmitter-containing presynaptic vesicle membranes, the increase in synaptophysin is potentially reflective of an increase in neurotransmission, that may in turn lead to improved spatial LTM (Frick & Fernandez, 2003).

The Wt mice that experienced EE exhibited relatively increased levels of inhibitory synaptic proteins in the cortex, and both increased excitatory and decreased inhibitory synaptic protein levels in the hippocampus. Cortical inhibition is vital in

coordinating network activity and maintaining cortical function (Isaacson & Scanziani, 2011). In contrast, the APP/PS1 mice from both housing conditions exhibited relatively high levels of both inhibitory and excitatory synaptic protein, suggesting an imbalance of the interplay between excitatory and inhibitory activity. A high level of Aβ is associated with aberrant excitatory network activity and compensatory inhibitory responses (Palop & Mucke, 2010). Aβ may affect excitatory and inhibitory synapses differently, thereby creating complex imbalances in neuronal circuit activity.

While both AD and EE induce widespread alterations to the brain, in AD some brain regions are more affected than others. One notable finding of the present study was the increase in the number of synaptophysin labelled puncta within the CA1 region of EE APP/PS1 mice. Consistent with these findings, CA1 activity has been found to increase when rodents are exposed to novel environments (Nitz & McNaughton, 2004; Cracchiolo et al., 2007; Csicsvari, O'Neill, Allen, & Senior, 2007; Karlsson & Frank, 2008). Similarly, an increase in CA1 synaptic density in healthy rodents following EE has been previously reported (Moser, Trommald, & Andersen, 1994; Moser, Trommald, Egeland & Andersen, 1997; Rampon et al. 2000; Malik & Chattarji, 2012).

Although the finding of increased CA1 synaptic connectivity following EE has been previously reported, the novelty here is that we found an increase in CA1 synaptic density in a model of Alzheimer's pathology, suggesting that this region retains capacity for experience-dependent plasticity in response to EE, despite the presence of accumulating Aβ neuropathology. An increase in the number of synapses in plaque-free brain regions in this mouse model at 12 months of age has been previously reported (West, Bach, Søderman & Jensen, 2009; Mitew et al. 2013a). While the CA1 region is not plaque free, Aβ plaques occupy only a negligible fraction of CA1 in FAD mouse models (Merino-Serrais, Knafo, Alonso-Nanclares, Fernaud-Espinosa, & Defelipe,

2011). Early-stage AD is characterised by attenuated synaptic plasticity, triggering a compensation response by the formation of new synapses in an attempt to preserve synaptic connectivity (King & Arendash, 2002; Selkoe, 2002; Boncristiano et al., 2005; Jansen et al., 2012). Here, the heightened number of synaptic contacts in the APP/PS1 mice may be a compensatory response to synaptic dysfunction (West et al., 2009). As EE targets CA1, it follows that the combination of this compensation response paired with stimulation of CA1 by EE would lead to an increase in synaptic density within this region.

The finding of an increase in hippocampal BDNF in APP/PS1 mice exposed to EE might suggest BDNF to be a mediator of the beneficial effects of EE. Stimulation of CA1 has been observed to increase hippocampal BDNF levels (Kealy & Commins, 2010). Moreover, hippocampal BDNF plays a critical role in learning and memory processes (Cowansage, LeDoux, & Monfils, 2010), potentially allowing for the APP/PS1 mice with unaltered Aβ plaque burden to demonstrate superior STM following 3 months of EE.

The findings presented here indicate EE initiated in mid-life promotes an array of beneficial effects in healthy ageing in terms of enhancing synaptic and cognitive health. Such findings are remarkable considering the lack of stimulation in early-life, suggesting later-life interventions may be able to overcome some of the negative effects of minimal stimulation earlier in life. However, when ageing also involves Aβ neuropathology, EE does not influence global positive effects as seen in healthy ageing. Although, EE after Aβ deposition has commenced, was associated with a more specific alteration, an increase in the number of synaptic contacts in the AD-vulnerable CA1 subregion, and an increase in hippocampal BDNF protein levels. These data demonstrate that plasticity processes are afforded by EE in specific brain regions

despite the presence of accumulating Aβ neuropathology. Hence, non-pharmacological interventions such as EE introduced during the preclinical stage of disease may promote compensatory mechanisms that enhance synaptic connectivity. Moreover, the findings presented in this Chapter demonstrates that an intervention targeted to mid-life based on EE has the potential to promote synaptic and cognitive health in healthy ageing.

This Chapter was designed to examine the effect of EE in mid-life, and following the deposition of Aβ in the FAD model, on subsequent Aβ load, and on cognitive and synaptic health. The findings presented in this Chapter offer no straightforward relationship between mid-life EE and these factors. Rather, there was a spectrum of differential effects produced from EE between healthy Wt and FAD model mice. Overall, mid-life EE in healthy Wt mice, was associated with a balance to inhibitory and excitatory synaptic connectivity, and positive effects on long-term memory. FAD mice exhibited more specific, and subtle effects, showing a shorter-term cognitive benefit in spatial short-term memory, and an increase in hippocampal BDNF and CA1 synaptic density. The finding of an increase in hippocampal BDNF and synaptic connectivity, is notable given the presence of accumulating Aβ neuropathology. The findings of this Chapter, of no alterations to subsequent amyloid pathology when EE is introduced after Aβ deposition are consistent with that of Verret and colleagues (2013). However, EE following Aβ-pathological onset has also been shown to reduce Aβ load (Herring et al., 2011). One limitation of research into EE, is that various EE paradigms are employed (Xu, Yu, Tan, & Tan, 2015), which potentially leads to differences in the amount of stimulation from the environment received. Here, the animals lived in a constant enriched environment. However, it is possible that a more intensive and novel form of stimulation may be required to buffer AD-related neuropathology.

Chapter 4

4. Environmental novelty exacerbates stress hormones and Aβ pathology in an Alzheimer's model

4.1 Introduction

Research has consistently demonstrated that the environment influences the brain both structurally and functionally over the lifetime (Sale, Berardi, & Maffei, 2009). In this respect, among modifiable factors that are associated with a reduced risk of developing dementia, is living a cognitively engaged life. Indeed, those who engage in high levels of cognitive activity have approximately half the risk of developing dementia than those who engage in low levels (Valenzuela & Sachdev, 2006). As discussed in Chapter 1 and 3, stimulation from the environment in animal models has a variable effect on Aβ pathological burden in transgenic mice expressing human FAD-related gene mutations, with reported decreased (Lazarov et al., 2005; Costa et al., 2007; Herring et al., 2011), no change (Arendash et al., 2004; Wolf et al., 2006; Cotel et al., 2012), or increased (Jankowsky et al., 2003; 2005) Aβ load. As demonstrated in the previous Chapter, EE from mid-life was not associated with alterations to subsequent Aβ plaque pathology in a FAD mouse model.

As discussed in Chapter 1, it is possible that more complex, and novel stimulation may be required to buffer AD-related neuropathology (Valenzuela & Sachdev, 2006). Therefore, in this Chapter, a potential 'dose' effect of EE was investigated, with added periodic augmentation of stimulation (EE+). In order to examine dose-effects of EE in mid to later-life, mice were raised in SH conditions until 6 months as in Chapter 3, and then randomly assigned to SH, EE, or an enhanced environmental enrichment (EE+) condition, where the animals remained until 12 months of age.

4.2 Methods

4.2.1 Animals and EE protocol

As for Chapter 3, male mice expressing chimeric mouse/human amyloid precursor protein (APP) and mutant human presenilin 1(PS1) on C57BL/6 background (APP/PS1; Jankowsky et al., 2004) and littermate wildtype (Wt) control mice were used. Animals were housed in standard housing (SH) conditions (see Chapter 2 for description) until 6 months of age. At 6 months, mice were randomly assigned to SH, EE, or EE+ conditions for the following 6 months. The mid-life EE protocol as detailed in Chapter 2 was followed for SH and EE housing paradigms. The EE+ condition involved the mice being exposed to a larger (36 x 49 x 22 cm) cage three times per week that contained novel enrichment objects (changed weekly; Chapter 2). Housing conditions were maintained until the 12-month end-point.

4.2.2 Tissue collection

Mice were terminally anaesthetized first with gas anaesthesia (isoflurane) followed by sodium pentobarbitone (100 mg/kg delivered intraperitoneally). For histological analysis, animals were perfused transcardially with 4% paraformaldehyde (PFA) in 0.1M phosphate buffered saline (PBS pH 7.4). Post-mortem brains were transferred to 18% then 30% sucrose solutions overnight. Brains were cut on a cryostat (Leica CM 1850) in 40µm coronal sections. APP/PS1 mice used for the quantification of human soluble $A\beta_{42,}$ were terminally anaesthetized as above, and perfused transcardially with PBS (0.1M). Post-mortem brains were removed and the cortex and hippocampus were dissected and immediately frozen in liquid nitrogen. Cortex and hippocampal samples were stored at -80°C for later analysis.

4.2.3 Aβ fibrillar plaque deposition

Thioflavin-S (Sigma-Aldrich) staining was performed in order to visualise fibrillar and dense-core Aβ plaques(Dickson & Vickers, 2001). Ten serial sections evenly spaced throughout the rostrocaudal axis of the brain from bregma 2.0 - ¯3.0 mm according to the stereotaxic mouse brain atlas (Paxinos & Franklin, 2008) were incubated in the solution (0.125% Thioflavin-S diluted in 60% absolute ethanol with 40% 0.01M PBS) for 3 minutes at room temperature, and washed for 2 x 1 minute in 50% absolute ethanol and 50% 0.01M PBS solution, followed by 3 x 10 minute washes in 0.01M PBS. Sections were mounted using Dako fluorescent mounting medium.

4.2.4 Image acquisition

In order to determine Aβ plaque load in the cortex and hippocampus, images were taken on a Leica DM fluorescence microscope on a 10x objective with NIS Elements imaging software. The left side of the cortex was imaged from the midline to the rhinal fissure from bregma 2.0 - ¯3.0 mm of 10 sections per animal. Images of the whole hippocampus were taken between bregma position -1.22 and -2.46 mm of 3-5 sections per animal. Aβ plaque load (percentage area Thioflavin-S positive) was calculated by random forest segmentation. Whereby, a custom plugin for ImageJ was applied to the images, which automatically segmented images as plaques or background pixels by random forest classification (Sommer et al., 2011; Chapter 2).

4.2.5 $A\beta_{42}$ ELISA

For the quantitation of human $A\beta_{42}$, a sandwich antibody ELISA was performed according to the manufacturer's instructions (KHB3441, Invitrogen). Duplicate cortex and hippocampus samples from APP/PS1 mice (n = 5/group) were diluted in the buffer

provided (1:50). Soluble human $A\beta_{42}$ levels were normalized to total protein levels and expressed as picogram of $A\beta_{42}$ content per milligram of total protein (pg/mg). Optical densities were read at 450 nm on a microplate reader (SpectraMax, Molecular Devices), and concentrations of $A\beta_{42}$ were determined by comparison to the standard curve using a 4-parameter algorithm.

4.2.6 Corticosterone ELISA

In order to determine if there were differences in levels of stress between the housing conditions and genotypes, blood was collected at time of perfusion by cardiac puncture, performed at the start of the dark period (3 p.m.). Serum samples were diluted at 1:100 in the buffer provided, and levels of serum corticosterone were measured by a commercial ELISA kit (ab108821; Abcam) according to manufacturer instructions. Optical densities were read at 450 nm on a microplate reader (SpectraMax, Molecular Devices), and concentrations of corticosterone were determined by comparison to the standard curve using a 4-parameter algorithm.

4.2.7 Statistical analysis

Statistical analyses were performed using independent t-test and two-way ANOVA using IBM SPSS (Version 20). A statistically significant two-way ANOVA was followed up by *post hoc* tests. Variables considered were genotype (Wt or Tg) and housing condition (SH, EE, or EE+). Values of $p < .05$ for differences between group means were classified as statistically significant. The magnitude of differences between the means were reported as Cohen's *d*.

4.3 Results

4.3.1 Body weight

No group differences were detected in body weight, F (2, 66) = 1.14, p = .33.

4.3.2 Mid-life EE+ was associated with increased fibrillar Aβ pathology in the hippocampus

Following the 6-month intervention, when mice were 12 months of age, it was hypothesised that exposure to the EE+ intervention would potentially be associated with a reduction in Aβ plaque burden. Fibrillar Aβ plaque load was analysed in the neocortex and hippocampus of APP/PS1 mice, and no significant alterations to Aβ plaque load in the neocortex were detected as a function of housing condition (Figure 4.1A). In contrast, there was a large (d = 1.08) and significant increase in hippocampal Aβ plaque load in APP/PS1 mice exposed to the EE+ condition (Figure 4.1B).

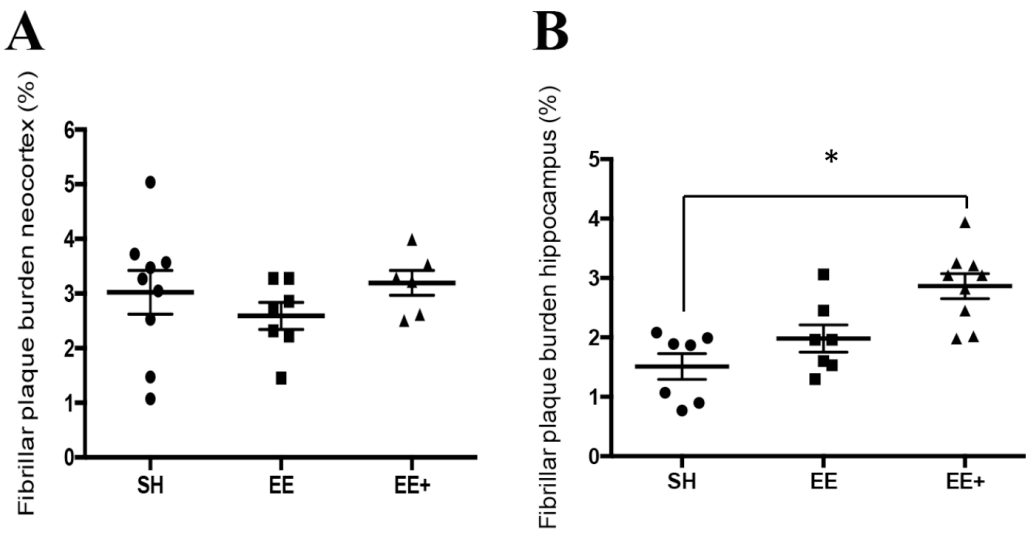

Figure 4.1. Fibrillar Aβ plaque load in neocortex and hippocampus in APP/PS1 mice exposed to differential housing. **A** Thioflavin-S staining of fibrillar and dense core Aβ plaques demonstrated no significant difference in plaque load in neocortex between the housing groups, $F_{(2, 19)} = 0.78$, $p = .47$. **B** A significant effect of housing was detected on plaque load in the hippocampus, $F_{(2, 19)} = 4.80$, $p = .02$. Bonferroni adjusted *post hoc* tests revealed APP/PS1 mice housed in EE+ had higher hippocampal Aβ plaque burden when compared to those in SH ($p = .02$). * $p<.05$, ** $p<.01$, *** $p<.001$.

4.3.3 Mid-life EE+ was associated with increased $A\beta_{42}$ in neocortex

Next, levels of soluble $A\beta_{42}$ in the neocortex and hippocampus were examined, and an overall significant effect of housing condition on $A\beta_{42}$ was found. APP/PS1 mice housed in EE+ showed a large ($d = 1.89$) and significant increase in $A\beta_{42}$ in neocortex, compared to mice from SH or EE (Figure 4.2A). However, in contrast to the finding of an increase in Aβ plaque deposition in the hippocampus, this effect was not observed for $A\beta_{42}$ levels in the hippocampus (Figure 4.2B). The data suggest that EE+ is associated with increases in Aβ production in the neocortex, and increased deposition of Aβ in the hippocampus.

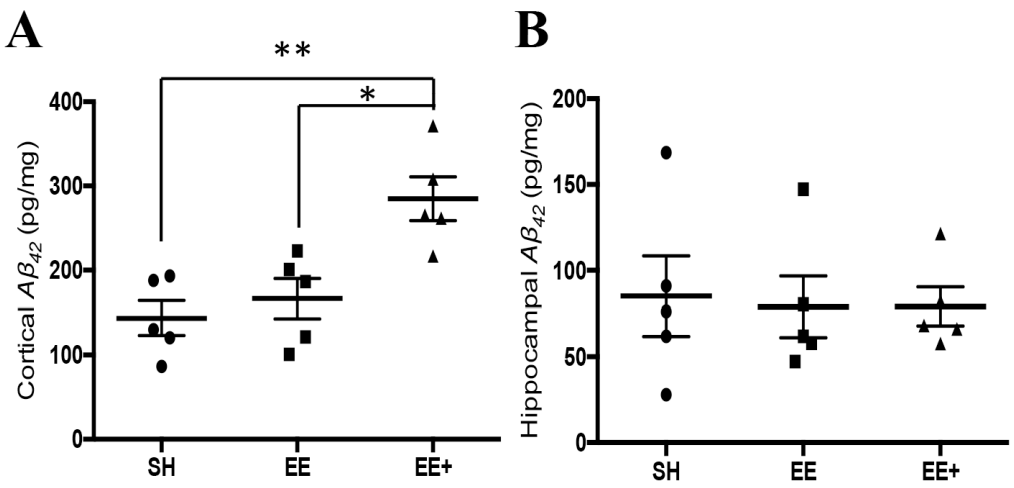

Figure 4.2. $A\beta_{42}$ levels in neocortex and hippocampus in APP/PS1 mice across differential housing conditions. **A** Overall, there was a significant effect of housing condition on cortical $A\beta_{42}$ levels, $F_{(2, 12)} = 10.41$, $p = .002$. Follow-up *post-hoc* tests were performed with Bonferroni correction applied. The EE+ group had significantly higher cortical $A\beta_{42}$ compared with both SH ($p = .003$) and EE ($p = .012$). **B** No significant differences were detected in hippocampal $A\beta_{42}$ levels between housing conditions, $F_{(2, 12)} = 0.04$, $p = .96$. * $p < .05$, ** $p < .01$, *** $p < .001$.

4.3.4 APP/PS1 mice demonstrated elevated levels of corticosterone

While the assumption had been that placing animals in an enhanced EE condition may have augmented cognitive and physical stimulation, it was also possible that this exposure had broader physiological effects that may have led to increased Aβ pathology, and that the FAD background also contributed to an unexpected reaction to the EE+ condition. Previous clinical studies have reported that people living with AD generally have higher blood-levels of the stress hormone, cortisol, when compared to healthy people (Rasmuson et al., 2001; Csernansky et al., 2006). However, whether the disease process itself alters normal stress physiology, or whether stress increases vulnerability to developing AD, is unknown. It is widely accepted that stress induces activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, which leads to a

subsequent release of cortisol in humans, or corticosterone in rodents, from the adrenal cortex (Nelson, 1972; Silverman & Sternberg, 2012).

Several studies, using experimental paradigms designed to induce stress, have demonstrated an accelerated onset, or faster rate of progression of AD, in FAD mouse models (Dong et al., 2004; Jeong et al., 2006; Carroll et al., 2011; Rothman et al., 2012; Baglietto-Vargas et al., 2015). Such evidence led to the hypothesis that the novelty and complexity conferred by the EE+ condition, elevated stress levels, which may in turn have increased Aβ pathological burden in APP/PS1 mice in EE+ housing. We first compared levels of the stress hormone, corticosterone, between healthy control Wt and APP/PS1 mice from the SH condition, in order to determine whether the presence of the APP/PS1 transgene was associated with elevated corticosterone. Corticosterone levels in blood serum were significantly increased in 12-month old APP/PS1 mice compared to healthy Wt mice, which represented a large effect (d = 1.09) (Figure 4.3). This finding supports the hypothesis of HPA axis dysfunction in AD.

4.3.5 Mid-life EE+ was associated with elevated corticosterone levels in APP/PS1 mice

Following the finding of elevated levels of corticosterone in APP/PS1 mice compared to Wt mice from SH, EE and EE+ conditions were included into the analysis. First, an overall significant interaction effect of genotype x housing on corticosterone levels was detected. Due to the APP/PS1 mice demonstrating increased corticosterone levels, genotypes were analysed separately in order to investigate housing effects. In Wt mice, there was no significant housing condition effect on corticosterone levels. However, there was a significant and large effect of housing condition for APP/PS1 mice. While the APP/PS1 mice from SH exhibited increased corticosterone levels relative to Wts, APP/PS1 mice from EE+ showed further elevated corticosterone levels,

being significantly higher compared to APP/PS1 mice from both the SH and EE conditions (d = 1.05) (Figure 4.3).

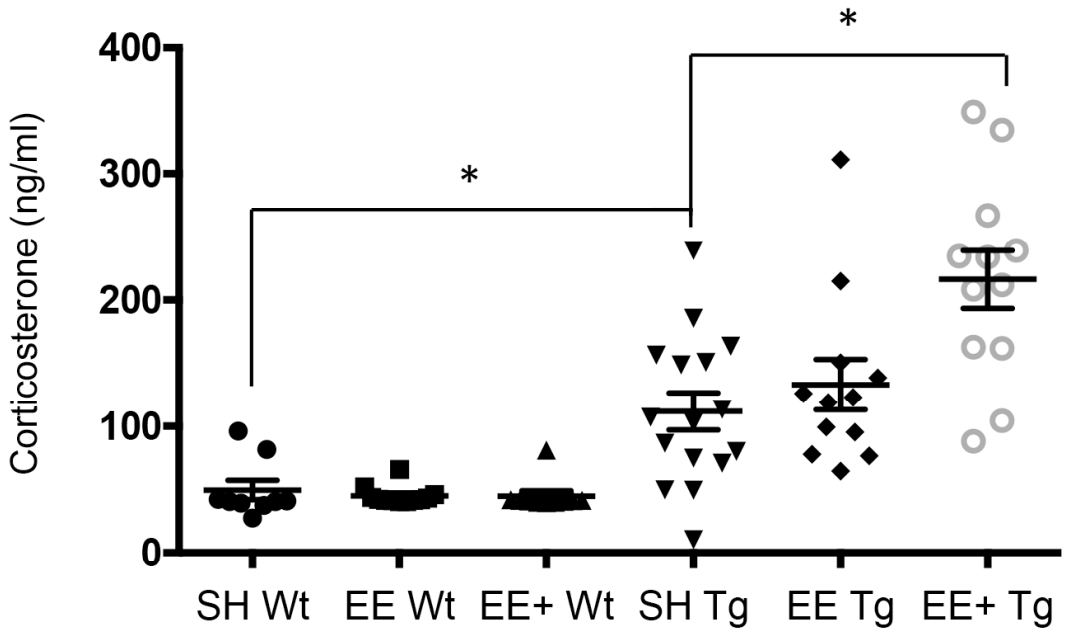

Figure 4.3. Differential corticosterone levels according to genotype and housing condition. APP/PS1 mice in SH had significantly higher levels of corticosterone in blood serum compared to healthy Wt control mice at 12 months, t (24) = 2.34, p = .03. There was a significant interaction effect of genotype x housing on levels of corticosterone, F (2, 66) = 3.76, p = .03. In Wt mice, there was no significant effect of housing on corticosterone, F (2, 28) = 0.31, p = .74. Housing condition produced a significant effect on corticosterone levels in APP/PS1 mice, F (2, 38) = 4.8, p = .01. Bonferroni adjusted *post hoc* tests revealed EE+ mice had significantly higher corticosterone levels compared to SH (p = .02). Moreover, EE+ mice had higher corticosterone levels than mice housed in EE (p = .049), however, this was no longer significant when Bonferroni correction was applied (p = .057). * p <.05, **p <.01, ***p <.001.

4.4 Discussion

This Chapter was designed to examine whether more complex, and novel stimulation, would confer a reduction to Aβ pathology relative to a more stable form of stimulation, as was employed in Chapter 3. In this study, the APP/PS1 mouse model showed elevated stress-hormone levels compared to Wt animals of a similar age. Periodic augmentation of EE conditions in APP/PS1 mice was associated with a deleterious effect on Aβ pathology, and this was associated with further heightened levels of corticosterone. In contrast, sustained exposure to regular EE conditions did not affect corticosterone levels or amyloid pathology, indicating a potential capacity to habituate to long-term changes in the housing environment. While APP/PS1 animals housed in EE+ had increased Aβ pathology along with elevated corticosterone, no significant behavioural differences were detected on the Y maze test of spatial short-term memory, or the Barnes maze test of spatial learning and long-term memory relative to APP/PS1 mice housed in SH or EE (data not shown). The finding of increased pathological burden without cognitive dysfunction may offer support for the cognitive reserve theory (Stern, 2012), however following up at later time points when neural resources are reduced, would be of interest.

Clinical studies have included reports of people living with AD to generally have higher levels of blood cortisol when compared to healthy people (Rasmuson et al., 2001; Csernansky et al., 2006). Moreover, elevated levels of corticosterone has been reported previously in a FAD mouse model (Guo et al., 2012), and Aβ accumulation has been associated with increased sensitivity of the HPA axis (Carroll et al., 2011). The present study confirms HPA axis dysfunction in an animal model of early AD. Several studies have demonstrated using experimental paradigms designed to induce

acute stress (e.g. by restraint, loud noise, and bright light) an accelerated onset, or faster rate of progression of AD, in FAD mouse models (Dong et al., 2004; Jeong et al., 2006; Carroll et al., 2011; Rothman et al., 2012; Baglietto-Vargas et al., 2015). However, in the current report, an association was detected between the periodic stimulation condition (EE+) and increased corticosterone.

APP/PS1 mice display Aβ pathology from 4-5 months of age (Jankowsky et al., 2004), in the hippocampus and neocortex, and associated damage to synapses (Mitew et al., 2013), and the hippocampus directly regulates the HPA axis (Herman et al., 1989; Jacobson & Sapolsky, 1991; Roozendaal et al., 2001). Green and colleagues (Green et al., 2006) speculated that, in AD, glucocorticoid feedback to the hippocampus may be lost, leading to an increased stress response. Thus, the increasing levels of glucocorticoid may further accelerate pathological processes, acting as a cycle of disease process progression.

Notably, Wt mice did not show increased corticosterone levels after exposure to EE+, and, thus, were not stressed by the periodic stimulation of the EE+ condition. In contrast, FAD gene-linked deposition of Aβ in the hippocampus and synaptic damage in transgenic animals may lead to initial dysregulation of the HPA-axis, which here, contributed to a heightened stress response following periodic stimulation in the EE+ condition. The periodic stimulation of EE+ may stimulate a harmful cascade, with subsequent increases in Aβ leading into an increased stress response to EE+, further increasing circulating corticosterone, and further increasing Aβ deposition within the hippocampus.

The findings of this study raises many implications. The finding that elderly who enter a nursing home demonstrate more rapid cognitive decline compared to those who remain in the community (González-Colaço-Harmand et al., 2014), together with

the findings of this study, raise questions into the effect of novel environment exposure for people with AD, and how this may alter, or hasten, disease progression. Moreover, the findings presented here bring into question cognitive interventions for the elderly, and for people living with AD in particular. An intervention aimed at delaying the progression of the clinical syndrome, dementia, is urgently needed given the rapidly increasing ageing population. As there are no current efficacious pharmacological treatments available, non-pharmacological interventions such as cognitive stimulation, training, and rehabilitation are of current interest. Such interventions have demonstrated various levels of efficacy, although modest-sized effects are typically reported (Choi & Twamley, 2013), and it appears that stress levels of the people living with AD undergoing such programs has not been examined. Moving forward, this will be an important point of investigation, as cognitive interventions for people living with AD may have limited beneficial effect, and may potentially induce stress leading to an increased progression of the disease process. Likewise, given that Aβ pathology may be present in the brain many years before overt dementia symptomology (Braak & Del Tredici, 2011), it will be important to examine the role that stress may have in facilitating further pathology, and that primary and secondary prevention strategies that involve cognitive stimulation are designed to minimise elevated stress.

Contrary to the hypothesis, the findings presented here demonstrated that a complex/novel environment was associated with an increase in Aβ pathological burden. Increased levels of the stress hormone, corticosterone, were detected in FAD mice, which were further elevated with novel environmental stimulation. The findings of Chapter 3 with together with these findings, suggest constant enrichment does not affect Aβ pathology, and has some differential beneficial effects on cognitive function. As discussed in Chapter 1, the neural mechanisms underlying this association are not well

understood, and are seldom investigated, with previous literature focusing on the effect on Aβ pathology. The data presented in Chapter 3 suggest EE to be associated with some promotion of synaptic connectivity within the hippocampus. However, other underlying mechanisms are yet to be elucidated. Emerging evidence suggests neuroinflammation to be key contributor to the pathogenesis of AD (Heneka et al., 2015), and it is not understood how EE may moderate this relationship. Given there are benefits of mid-life enrichment, the question was posed of whether late-life enrichment would too confer benefit. There is limited literature from both animal and human studies surrounding the effect of environmental stimulation in later-life, however, this is a critical life-phase to investigate given our rapidly ageing population. Even "normal" ageing is accompanied with a reduction in cognitive and mental capacity (e.g. Salthouse, 2012). Interventions aimed at protecting cognitive health in ageing are vital, as a preservation of cognitive health in ageing is associated with being less likely to have a premature death, depend on careers, live in a long-term care setting, or be disabled, or hospitalized (McGuire, Ford, & Ajani, 2006).

Chapter 5

5. The effect of late-life environmental enrichment on cognitive function and microglia

5.1 Introduction

As discussed in Chapter 1, the underlying mechanisms leading to a promotion of cognitive function by EE in transgenic models of AD pathology and in models of healthy ageing, are not well understood. There have been some reports that for healthy animals, the benefical effects of EE may involve modulation by the brain's immune cells (Ziv et al., 2006; Choi et al., 2008; Ziv & Schwartz, 2008; Vukovic et al., 2012).

Microglia are the resident immune cells in the CNS, and constantly survey their environment for pathogens, apoptotic cells, and foreign material (Streit et al., 2004). There is emerging evidence of microglia being critically involved in the elimination and refinement of synaptic connections in the healthy brain. Microglial processes constantly contact axons, dendritic spines, and synapses (Stevens et al., 2007; Paolicelli et al., 2011; Schafer et al., 2012). Recently, microglia have been found to prune synapses, where extraneous synapses are removed, and meaningful synapses remain intact (Tremblay, Lecours, Samson, Sánchez-Zafra, & Sierra, 2015), a process that continues throughout adulthood, and vital to the maintenance of normal brain function (Chen et al., 2014). The dynamics of microglial processes have been found to be regulated by sensory experience and neuronal activity (Wake, Moorhouse, Jinno, Kohsaka, & Nabekura, 2009; Tremblay, Lowery, & Majewska, 2010). Such evidence has led to the assumption that microglial contact with synapses may be regulated by complex cognitive activity, such as learning and memory processes (Morris, Clark, Zinn, & Vissel, 2013) engaged in by EE.

Microglia, may also have a key role in AD pathogenesis. Two recent genome-wide association studies identified that a polymorphism in the triggering receptor

expressed in myeloid cells 2 (TREM2) gene, which is associated with decreased phagocytosis and an increased pro-inflammatory reactive phenotype of microglia (Guerreiro et al., 2013), is associated with a significant increased risk for developing AD (Guerreiro et al., 2013; Jonsson et al., 2013). Moreover, microglia frame dense-core Aβ plaques (Itagaki et al., 1989), and exhibit an altered phenotype to that of a healthy brain, leading to extensive research efforts focused on the role of microglia in AD pathogenesis.

Experimental studies have demonstrated microglia are able to engulf Aβ and may act as a primary defence against Aβ accumulation (Prokop, Miller, & Heppner, 2013). Microglia help to eliminate Aβ through phagocytosis, however, on the other hand, facilitate Aβ accumulation through the release of inflammatory factors and neurotoxic proteases (Ard, Cole, Wei, Mehrle, & Fratkin, 1996; Majumdar, Capetillo-Zarate, Cruz, Gouras, & Maxfield, 2011). Microglia secrete neurotoxic molecules, which is associated with an exacerbation of Aβ pathology (Combs, Johnson, Cannady, Lehman, & Landreth, 1999; Combs, Johnson, Karlo, Cannady, & Landreth, 2000). However, microglia also secrete neuroprotective cytokines in the AD brain (Meda et al., 1999). Whether microglia exert beneficial or detrimental effects in the AD brain, may be dependent on the stage of pathology. It has been proposed that microglia are beneficial in preventing plaque formation in early stages of disease, however, in later stages microglia demonstrate detrimental effects (Prokop et al., 2013).

Whether microglia serve a protective or detrimental role in AD pathology, may be dependent on ageing. Microglia undergo morphologic changes during aging, demonstrated by an increased soma size, and shorter, more sparse, and dystrophic processes (Streit, 2006; Flanary et al., 2007). These morphological alterations may confer functional deficits, as observed in *in vivo* and *in vitro* experiments, leading to

ineffective phagocytosis of Aβ (Sierra et al., 2007; Floden & Combs, 2011) which may provide one mechanism for AD being most typically, a disease of ageing.

Depending on the immune challenge, microglia exhibit differing phenotypes, and orchestrate differing functions within the CNS from inflammatory responses, to assisting in brain repair (Southam, Vincent, & Small, 2016). 'Resting' microglia are morphologically characterized by a small-sized soma, and fine processes that constantly survey, sense, and screen their environment (Streit et al., 2004; Nimmerjahn et al., 2005). In AD, microglia frame dense-core Aβ plaques, and show an altered morphology, typically thickened, dystrophic processes, and increased soma size (Itagaki et al., 1989), and are observed to have an 'activated', pro-inflammatory phenotype (Perlmutter et al., 1990). 'Activated' microglia clear foreign material through cytotoxic and phagocytic mechanisms throughout the CNS (Ransohoff & Perry, 2009). However, microglia can exhibit opposing activation states, conferring alternate inflammatory responses. Briefly, an M1 state is associated with defence against infection and is considered pro-inflammatory. In contrast, an M2 state is associated with an attenuation of inflammation (Southam, Vincent, & Small, 2016).

There has been limited work on how microglia may be involved in the relationship between EE, cognitive function, and Aβ neuropathology. One recent study (Xu et al., 2016) demonstrated EE-induced morphological and functional alterations to microglia in *in-vivo* and *in-vitro* experiments, and an associated prevention of an inflammatory response to soluble oligomeric Aβ. Notably, these experiments were performed on relatively young mice (approximately 3 months of age), and the impact that EE may have on senescent microglia in models of healthy ageing, and in AD-associated pathological ageing, is a concept relatively unexplored.

Microglia are critical in both brain injury and in repair, and may, therefore, represent an underlying mechanism for the positive functional effects of EE on brain function and in resilience to pathology. In the previous Chapters, EE from mid-life up until 12 months of age was investigated. In this study, the EE intervention was extended to begin at 12 months of age, when APP/PS1 mice have established pathology, and terminated at 18 months, in order to examine later-life effects of EE. In this Chapter, the influence of EE on microglia was investigated in an APP/PS1 mouse model of established Aβ neuropathology. Previous studies on young rodent models have reported an increase in microglia quantity following EE. In the current study on a model of advanced AD pathology, in which microglia are thought to have detrimental effects, it was hypothesised that EE would be associated with a reduction to microglia quantity occupying regions important for learning and memory processes, the neocortex and hippocampus, and an associated rescue of cognitive function.

5.2 Materials and Methods

5.2.1 Environmental enrichment paradigm

Male transgenic APPswe, $PSEN1_{dE9}$ (APP/PS1) were used for the present study (described in Chapter 2). All animals lived in standard housing (SH) conditions which comprised group housing (4-5 mice per 30 x 30 x 14 cm cage), *ad Libitum* access to food and water, an igloo, one small wooden stick and one tissue from weaning until 12 months of age. From 12 months, APP/PS1 (n = 15) and littermate wildtype control (Wt; n = 17) mice were randomly assigned to SH or EE conditions (Late-life EE paradigm, described in Chapter 2). Housing conditions were maintained until the 18-month end-point.

5.2.2 Cognitive assessment

The cognitive assessment protocol was followed as for Chapter 2. In this study, mice were tested at 12-month baseline (before assignment to differential housing conditions), and again at 18 months. All mice were tested on the Y maze task of spatial short-term memory, and the Barnes maze test of learning and long-term memory, as outlined in Chapters 2 and 3.

5.2.3 Tissue preparation

Following the final day of behavioural testing, mice were weighed and then terminally anaesthetized first with gas anaesthesia (isoflurane) followed by sodium pentobarbitone (100 mg/kg delivered intraperitoneally). Animals were perfused transcardially with 4% paraformaldehyde (PFA) in 0.1M phosphate buffered saline (PBS pH 7.4). Brains were then dissected out and transferred to 18% then 30% sucrose solutions overnight. Brains for histology were serially sectioned on a cryostat (Leica CM 1850) in 40μm coronal sections. Sections used for analysis were from bregma 2.0 - ¯3.0 mm according to the stereotaxic mouse brain atlas (Paxinos & Franklin, 2008).

5.2.4 Immunohistochemistry and histological staining

5.2.4.1 Thioflavin-S

Thioflavin-S (Sigma-Aldrich) staining was performed in order to visualise fibrillar and dense-core Aβ plaques (Dickson & Vickers, 2001). Ten serial sections evenly spaced throughout the rostrocaudal axis of the brain from bregma 2.0 - ¯3.0 mm according to the stereotaxic mouse brain atlas (Paxinos & Franklin, 2008) were incubated in Thioflavin-S and the protocol as outlined in Chapter 2 was followed.

5.2.4.2 MOAB-2

The MOAB-2 antibody recognises human fibrillar, as well as unaggregated, oligomeric $A\beta_{42}$ (Youmans et al., 2012). Antigen retrieval by formic acid treatment was performed in order to enhance immunoreactivity (Kai et al., 2012). As for Thioflavin-S staining, ten sections evenly spaced throughout the rostrocaudal extent of the brain (bregma 2.0 - ¯3.0 mm), and the protocol as outlined in Chapter 2 was followed.

5.2.4.3 Iba1

The Iba1 antibody recognises the ionized calcium binding adaptor molecule 1, and immunolabells microglia (Ito et al., 1998). Three sections at approximately bregma 1.045, -1.855, and -2.88 mm were incubated in formic acid, and immunolabelled with the Iba1 antibody following the protocol in Chapter 2.

5.2.5 Image acquisition and analysis

In order to determine microglia density, imaging of Iba1 labelling was performed on a Perkin-Elmer Ultraview VOX confocal imaging system with Volocity 6.3 imaging software. Images of neocortex from the corpus collosum to the rhinal fissure and whole hippocampus were acquired using a 20x objective and constant laser and exposure settings. In order to determine Aβ plaque load in the neocortex and hippocampus, images of Thioflavin-S and MOAB-2 stained brain sections were obtained with a Leica DM fluorescence microscope on a 10x objective and NIS Elements imaging software. Ten sections evenly spaced from the rostral to caudal extent of the neocortex from bregma position 2.0 to -3.0 mm were imaged for MOAB-2 and Thioflavin-S Aβ plaque load. The left side of the neocortex was imaged from the midline to the rhinal fissure of 10 sections per animal. Images of the whole

hippocampus were taken between bregma position -1.22 and -2.46 mm of 3-5 sections per animal.

5.2.6 Random forest classification for the analysis of histology

Images of brain sections stained with Thioflavin-S, or labelled with MOAB-2, and Iba1, were processed to 8-bit greyscale images, and segmented with a custom plugin for ImageJ (See Chapter 2).

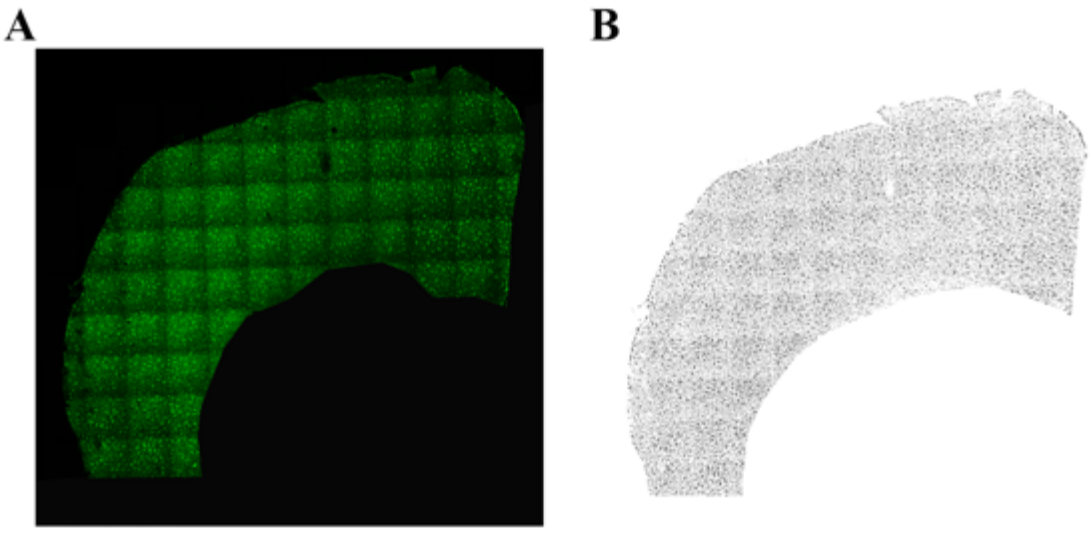

Figure 5.1. Example of random forest segmentation. **A** One hemisphere of coronal mouse brain section immunolabelled with the Iba1 antibody. **B** Resulting image following random forest segmentation.

5.3 Results

5.3.1 Body weight

All mice were weighed at the completion of the differential housing period, and a one-way ANOVA was conducted in order to determine if there were any significant weight differences between groups that could act as a confounding factor. There were no significant differences across housing condition or genotype on body weight, $F(1, 29) = 0.27$, $p = .4$.

5.3.2 Y maze

Healthy Wt and APP/PS1 (Tg) mice were tested for spatial short-term memory function on the Y maze at 12 months, before being assigned to differential housing. An independent t-test demonstrated no significant difference in time spent in the novel arm of the maze between genotypes $t(30) = 0.16$, $p = .88$. Following the 6-month differential housing period, repeat testing was performed, a significant housing x genotype interaction was detected, $F(1, 28) = 7.79$, $p = .009$. A separate independent t-test was performed for Tg animals, which showed Tg mice housed in EE spent significantly more time in the novel arm of the maze relative to Tg mice housed in SH, $t(13) = 8.1$, $p < .001$.

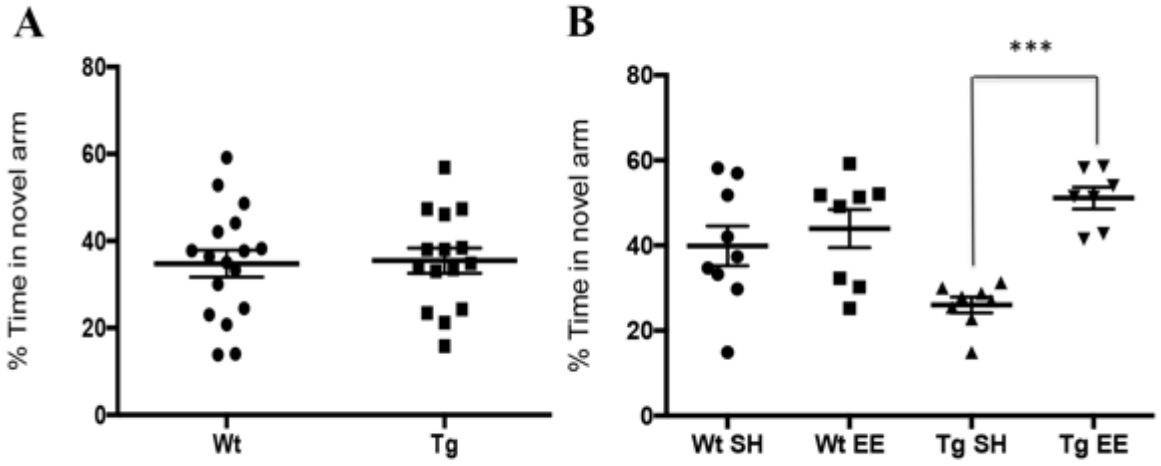

Figure 5.2. Y maze performance as mean percentage of time spent in the novel arm of the maze (± SEM). **A** 12-month baseline testing revealed no significant difference in the percentage of time spent in the novel arm of the maze, t (30) = 0.16, p = .88. **B** Y maze testing following the 6-month differential housing period (18 months of age) revealed housing-dependent performance differences for APP/PS1 (Tg) mice, where those in EE on average spent more time in the novel arm of the maze, t (13) = 8.1, p < .001.

5.3.3 Barnes maze

Following the 6-month differential housing period, when animals were 18 months of age, rate of learning was measured by performance on the Barnes maze over 7 days. There was no significant learning effect across housing x genotype, F (1, 28) = 0.05, p = .82. No significant effect of housing x genotype was detected on the long-term memory trial of the Barnes maze, F (1, 28) = 2.52, p = .12. However, an independent t-test demonstrated a significant housing effect for Wt mice, where those housed in EE had on average, a significantly reduced latency to reach the escape on the long-term memory trial compared to those from SH, t (15) = 3.26, p = .005.

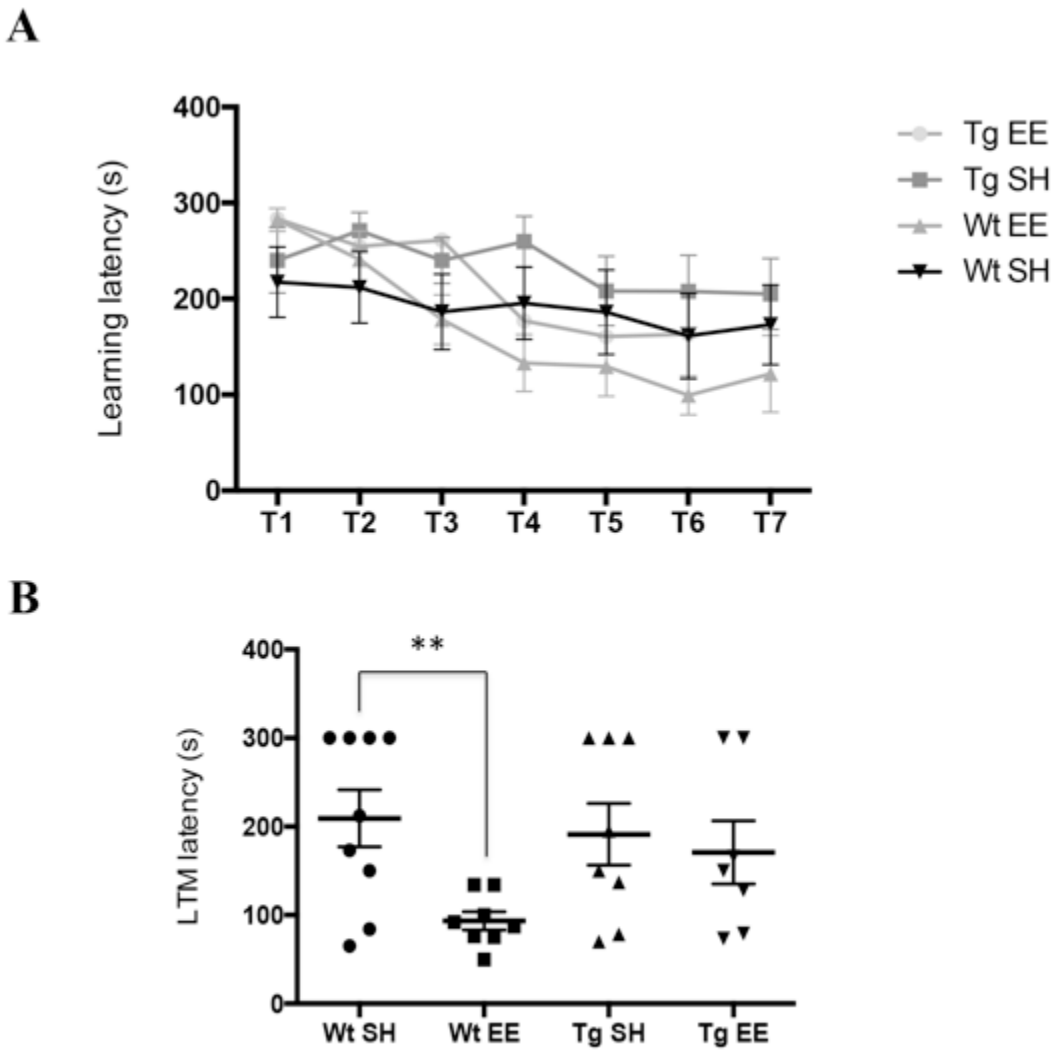

Figure 5.3 Barnes maze performance following differential housing, showing mean latency to reach the escape box (± SEM). **A** No significant differences in rate of learning were detected between the groups on the 7-day trial Barnes maze, F (1, 28) = 0.05, p = .82. **B** Only healthy Wt mice demonstrated performance differences as a function of housing, in which Wt mice had on average a significantly lower latency to reach the escape compared to Wt mice from SH, t (15) = 3.26, p = .005

5.3.4 Aβ load

Fibrillar Aβ plaques were visualised with the Thioflavin-S stain, and no significant differences between the housing conditions were detected in Thioflavin-S positive plaque load in neocortex, t (13) = 0.73, p = .48, or in hippocampus, t (13) = 0.81, p = .44. MOAB-2 positive labelling was not significantly different according to housing condition in neocortex, t (13) = 0.12, p = .91, or in hippocampus, t (13) = 0.84, p = .42.

Table 5.1. Aβ plaque load following late-life differential housing

	SH n = 8 M (SEM)	EE n = 7 M (SEM)
Thioflavin-S (% load neocortex)	3.68 (0.19)	3.67 (0.16)
Thioflavin-S (% load hippocampus)	2.74 (0.17)	2.98 (0.25)
MOAB-2 (% load neocortex)	26.43 (1.44)	26.84 (3.35)
MOAB-2 (% load hippocampus)	16.88 (2.34)	19.29 (1.55)

5.3.5 Microglia

In order to examine the effect of EE and genotype on microglia, brain sections were immunolabelled with the antibody Iba1, and the percentage area of labelled microglia (% microglia) occupying the neocortex and hippocampus were compared across housing and genotype groups. First, independent of housing, there was a significant genotype effect on the % microglia, with Tg mice having a greater percentage area of microglia within the neocortex ($t_{(8)}$ = 2.95, p = .02), and hippocampus ($t_{(8)}$ = 3.93, p = .004), relative to Wts (Figure 5.4). However, overall, no significant housing x genotype effects were detected on the percentage area of

neocortex occupied by microglia ($F_{(1, 16)} = 1.53$, $p = .23$), or that of the hippocampus ($F_{(1, 16)} = 2.98$, $p = .10$). Of note, is that a significant effect of microglia was detected for SH mice between genotypes, where Tg mice had a significantly higher percentage area occupied by microglia in neocortex ($t_{(8)} = 2.95$, $p = .02$) and hippocampus ($t_{(8)} = 3.93$, $p = .004$) relative to the Wts. However, this genotype effect was not detected for EE mice in the neocortex ($t_{(8)} = 1.29$, $p = .23$), or in the hippocampus ($t_{(8)} = 1.02$, $p = .34$). Although no statistically significant effects were detected across housing conditions, a large-sized effect was found for Tg mice between housing conditions in area occupied by microglia in neocortex ($d = 0.86$) and in hippocampus ($d = 1.09$), and a small effect was detected between the housing conditions for Wt mice in neocortex ($d = 0.35$), and a moderate effect for the hippocampus ($d = 0.53$) (Figure 5.4).

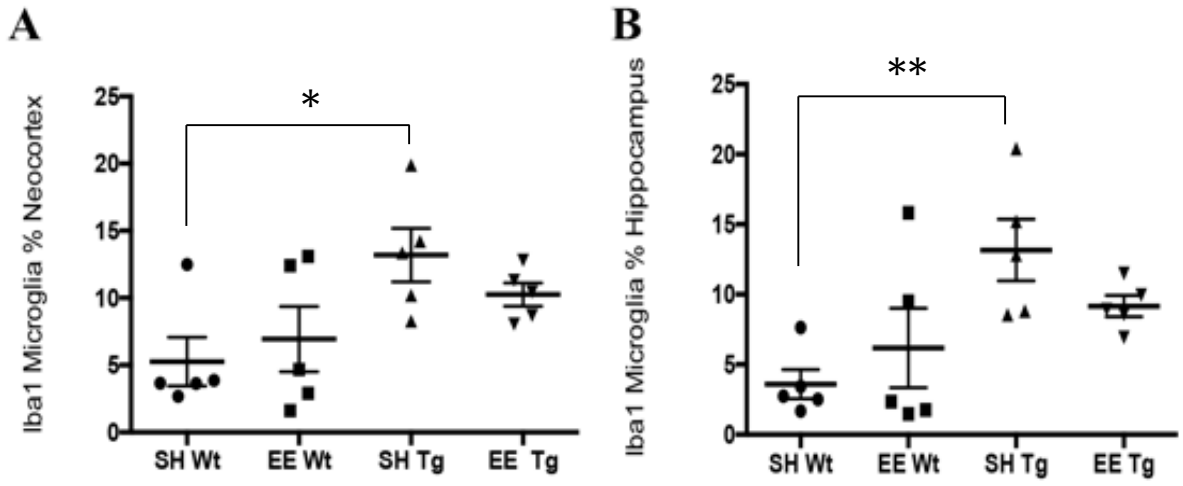

Figure 5.4. Percentage area occupied by Iba1 immunolabelled microglia. **A** APP/PS1 (SH Tg) mice had an increased percentage area of microglia in neocortex relative to Wt (SH), t (8) = 2.95, $p = .02$. **B** APP/PS1 (SH Tg) mice had an increased percentage area of microglia in hippocampus relative to Wt, t (8) = 3.93, $p = .004$.

5.3.6 The effect of microglia on cognitive function

There were some noteworthy correlations detected between % microglia and cognitive function. Large-sized correlations were detected between percentage area of microglia in neocortex and hippocampus and Y maze performance (r = 0.66*; 0.52 respectively), where a greater percentage of microglia predicted better Y maze performance when all groups were included in the analysis. However, considering EE has differential effects on the groups, separate analyses were also conducted on each housing/genotype group. Wt mice from SH showed a large-sized positive correlation between Y maze performance and % microglia (neocortex r = 0.86; hippocampus r = 0.75). Wt mice from EE also displayed a positive correlation between microglia density and Y maze performance (neocortex r = 0.54; hippocampus r = 0.39). In contrast, Tg mice from both housing conditions demonstrated negative correlations between Y maze performance and % microglia. Tg mice from SH had a moderate correlation between % microglia and Y maze performance (neocortex r = ¯0.33; hippocampus r = ¯0.48), and large-sized correlations were detected for Tg EE mice (neocortex r = ¯0.70; hippocampus r = ¯0.55).

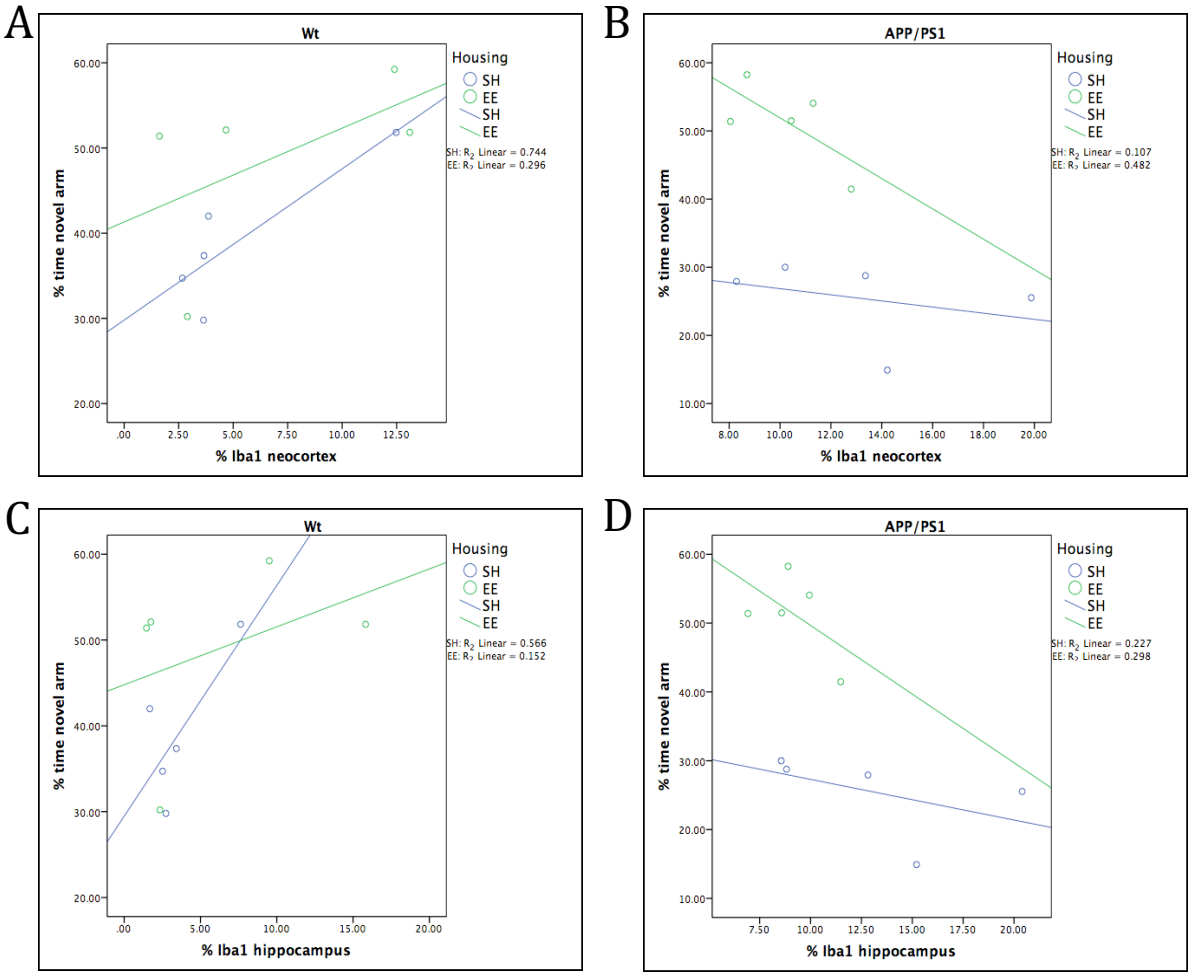

Figure 5.5. Graphical presentation of linear correlations between percentage area occupied by microglia and Y maze performance. **A** Y maze performance and % microglia in neocortex for Wts (SH: $r = 0.86$; EE $r = 0.54$). **B** Y maze performance and % microglia in neocortex for APP/PS1 mice (SH $r = -0.33$; EE $r = -0.70$). **C** Y maze performance and % microglia in hippocampus for Wt mice (SH $r = 0.75$; $r = 0.39$). **D** Y maze performance and % microglia in hippocampus for APP/PS1 mice (SH $r = -0.48$; $r = -0.55$).

Given the APP/PS1 mice demonstrated differential Y maze performance as a function of housing condition, a linear hierarchical regression analysis was performed in order to partial out the influence of housing condition and Aβ load, to investigate the underlying relationship between microglia density and Y maze performance. The analysis conducted on APP/PS1 mice produced a significant model for predicting Y maze performance by housing condition, $F(1, 8) = 44.54$, $p = < .001$, with housing

condition explaining 84.8% of the variance in Y maze performance. Aβ load was entered second into the model (including MOAB-2 and Thioflavin-S load in neocortex and hippocampus) and accounted for an additional non-significant 4.5% of the variance in Y maze performance above that of housing condition, $\Delta F\ (4, 4) = 0.42$, $p = .79$. Microglia (% Iba1labelled microglia in neocortex and hippocampus) was added third into the analysis, and accounted for an additional 8.2% of the variance in short-term memory function as measured by Y maze performance above housing condition and Aβ load, however, this was not statistically significant, $\Delta F\ (2, 2) = 3.22$, $p = .24$.

A separate linear hierarchical regression analysis was performed for the Wt group on long-term memory performance, as performance levels were different according to housing condition on this task. Housing condition accounted for 35.9% of the variance in long-term memory performance, which was not statistically significant, $F\ (1, 8) = 4.47$, $p = .06$. Entering % microglia at the second step of the model, accounted for an additional 9.9% of the variance in long-term memory performance on the Barnes maze, $\Delta F\ (2, 6) = 0.55$, $p = .61$.

5.4 Discussion

Consistent with the findings presented in Chapter 3 on 12-month old mice, EE was not associated with a reduction to Aβ neuropathological burden at 18 months, following 6 months of exposure to EE conditions. The findings of Chapter 3 demonstrated mid-life EE to be associated with improvements to cognitive function differentially according to genotype. Late-life EE in the current Chapter was also associated with cognitive improvement, which is notable considering the animals lived in an environment conferring very minimal stimulation for the first 12 months of life. Moreover, late-life EE was associated with a restoration of short-term memory function in APP/PS1 mice to the level of healthy Wt mice. The mechanisms underlying the association between EE and heightened cognitive function remain to be elucidated, and considering microglia are critically involved in repair of the CNS, they are potentially key to this association.

APP/PS1 mice housed in SH demonstrated an increase in the area occupied by microglia in both neocortex and hippocampus relative to Wts in SH, an effect reported on previously in the human AD brain (McGeer, Itagaki, Tago, & McGeer, 1987; Carpenter, Carpenter, & Markesbery, 1993) and in FAD mouse models (Janelsins et al., 2005; Meyer-Luehmann et al., 2008; Rodríguez, Noristani, & Verkhratsky, 2015). Microglia are thought to have a key role in the pathogenesis of AD, and may be one critical factor in the link between EE and Aβ pathology. If an EE paradigm promotes microglial health, enabling microglia to phagocytose Aβ effectively, this may account for a reduction in Aβ load found in some studies (Lazarov et al., 2005; Costa et al., 2007; Herring et al., 2011). In the current study, late-life EE had differential, and complex effects on microglia.

APP/PS1 mice from EE did not show this significant increase in percentage area occupied by microglia relative to Wts, as was observed in the SH condition. Previous evidence suggests the observed increase in microglia density in AD, is due to an altered, enlarged morphology of microglia, as opposed to an increase in proliferation (Serrano-Pozo, Gómez-Isla, Growdon, Frosch, & Hyman, 2013). Therefore, the finding of APP/PS1 mice from EE having a relatively lower percentage area occupied by microglia relative to APP/PS1 mice from SH (large-sized effect), may reflect the EE mice having less microglia with an enlarged, pro-inflammatory phenotype (Streit, Xue, Tischer, & Bechmann, 2014).

As APP/PS1 mice demonstrated an increase in short-term memory function following EE, a hierarchical regression analysis was conducted in order to examine the influence that microglia have in this association. Each measure of Aβ load was entered into the model in one step, and accounted for 4.5% of the variance in short-term memory function. Microglia (% area) accounted for an additional 8.2% of the variance in short-term memory function, which was not statistically significant, although notable considering the effect is larger than that of Aβ load. Despite no detection of statistically significant effects between microglia and cognitive function, moderate to large effect sizes were detected. APP/PS1 mice demonstrated a negative association between area occupied by microglia and short-term memory function, that is a greater percentage of microglia predicted lower performance levels. This may be reflective of the alterations in the phenotype of microglia observed in APP/PS1 mice, from 'resting' to an 'activated' state. The negative association between microglia and cognitive function in APP/PS1 mice may be due to them possessing a phenotype more representative of a reactive inflammatory phenotype, an effect particularly apparent in the SH mice. On the other hand, Wt mice demonstrated a positive association between microglia and

cognitive function, which is hypothesised to be due to having more "healthy", resting microglia (Streit et al., 2004; Nimmerjahn et al., 2005). Future studies investigating microglial phenotypes will address this hypothesis.

Microglia in the aged or diseased brain switch their phenotype to "primed" in which they produce neurotoxic molecules in response to inflammatory signals (Perry, Cunningham, & Holmes, 2007). This alteration in phenotype may offer one potential mechanism to explain the link between activated microglia and cognitive impairment. A reduction in cytokine and chemokine levels, indicative of a reduced pro-inflammatory phenotype of microglia, has been observed in healthy rats following EE (Williamson, Chao, & Bilbo, 2012). In addition, TNFα, a cytokine thought to be a key regulator or neuroinflammation (e.g. Holmes et al., 2009), was reported to be decreased by EE, and resulted in a neutralising effect on neuroinflammation (Xu et al., 2016). In the current study, a positive correlation was observed in Wt animals between microglia and memory performance, which may be representative of more microglia exhibiting protective functions, such as neutralising inflammation. Alternatively, activated microglia such as in AD, secrete interleukin-1β (IL-1β). Elevated levels of IL-1β in the hippocampus has been associated with cognitive impairment (Williamson, Sholar, Mistry, Smith, & Bilbo, 2011), which may offer a mechanism underlying the negative correlation between microglia and memory performance in APP/PS1 mice.

In conclusion, the findings of this study suggest that late-life EE is associated with increased short-term memory function in APP/PS1 mice independent to alterations to Aβ load. A strong, genotype-dependent association between microglia and short-term memory function was detected, adding support for the putative relationship between microglia and cognitive function. APP/PS1 mice were found to have an increase in percentage area occupied by microglia in the hippocampus and neocortex relative to

healthy ageing Wt mice, however, EE altered this effect, resulting in no genotype differences. This effect was hypothesised to reflect an alteration in phenotype, in which APP/PS1 mice from EE may exhibit a less reactive, pro-inflammatory microglial phenotype. Overall, microglia appear to play an important role in the association between EE and cognitive protection in the presence of Aβ neuropathology. Further studies investigating the phenotype of microglia in a FAD model following EE are warranted, to further elucidate how the brain's immune cells modulate stimulation from the environment and cognitive protection.

Chapter 6

6. Final discussion

6.1 Aim 1: Investigate the effect of mid-life environmental enrichment on cognitive and synaptic health in healthy and Alzheimer's disease-associated pathological ageing

Key findings

A wealth of research literature offers support for stimulation from the environment beginning in early-life to have beneficial effects on cognitive ageing in later-life, potentially by building a reserve against pathological damage, delaying cognitive morbidity to later years. However, there have been limited research efforts focused on examining cognitive intervention targeted at mid-life. Moreover, whether cognitive stimulation can enhance the compensatory mechanisms that promote synaptic connectivity for existing pathology, is unknown.

The results of this study demonstrated that EE initiated after Aβ plaque deposition has begun does not modify the course of subsequent Aβ pathology. Mid-life EE was associated with some cognitive protection to short-term memory following 3 months of EE, in APP/PS1 mice, and an increase in synaptic contacts in the hippocampal subregion CA1, as well as elevated levels of hippocampal BDNF. The findings regarding APP/PS1 mice are notable, given the lack of stimulation in early-life, and despite increasing amyloid levels associated with the transgene. Synapse loss is a strong correlate of cognitive decline in AD (DeKosky & Scheff, 1990; Terry et al., 1991; Scheff & Price, 2006; Scheff, Price, Schmitt, Dekosky, & Mufson, 2007; Arendt, 2009). The findings of this study demonstrate that mid-life environmental stimulation

may be an efficacious intervention in early-stage AD by promoting synaptic health and delaying cognitive deterioration. In healthy Wt mice, mid-life EE was associated with enhanced long-term memory function, and relatively increased levels of inhibitory synaptic proteins in the neocortex, and both increased excitatory and decreased inhibitory synaptic protein levels in the hippocampus, which may potentially reflect a more harmonious balance in the interplay between excitatory and inhibitory activity relative to SH or APP/PS1 mice. Overall, the findings of this study demonstrate that mid-life EE is associated with the promotion of synaptic and cognitive health in both healthy, and AD-associated pathological ageing.

6.2 Aim 2: Examine the effect of complex, novel environmental enrichment on Aβ neuropathology

Key findings

Given the findings of study 1 (Chapter 3), that mid-life EE was not associated with reduced Aβ neuropathology, a 'dose' effect of EE on amyloid pathology was investigated. This is particularly important to elucidate given the heterogeneity in findings from previous reports (Table 1). Furthermore, as acknowledged in Chapter 1, there is evidence of stress-hormone levels being associated with Aβ pathology (Dong et al., 2004; Jeong et al., 2006; Carroll et al., 2011; Rothman et al., 2012; Baglietto-Vargas et al., 2015), however, it not a factor controlled for in EE studies on FAD mice. The first key finding of this study, was that APP/PS1 mice demonstrated increased levels of the stress hormone corticosterone, relative to healthy Wts. When exposed to periodic enhanced cognitive stimulation, these animals demonstrated further heightened levels of corticosterone as well as increased Aβ pathology. Hence, these findings suggest AD may be associated with HPA axis dysfunction, causing stimulating

environments to become stress-inducing, leading to a glucocorticoid-pathology cycle contributing to further Aβ release and plaque formation. This finding suggests that stimulation-based interventions for AD need to be designed to minimise a stress response that may exacerbate brain pathology.

6.3 Aim 3: Explore the effect of late-life environmental enrichment on cognitive function and microglia

Key findings

Ageing-related alterations to microglia are increasingly recognised as a vital contributor to AD pathogenesis. The aim of this study was to assess whether EE initiated in later life, and at a stage of advanced Aβ pathology would lead to positive cognitive effects as demonstrated in the mid-life cohort, and whether microglia are involved in this relationship. The key findings of this study were that late-life EE did not alter amyloid pathology, however, was associated with enhanced long-term memory performance in Wts and restoration of short-term memory function in APP/PS1 mice. APP/PS1 mice in SH demonstrated an increase in the percentage area of neocortex and hippocampus occupied by microglia compared to WTs in SH. However, with EE there was no genotype effect on microglia, which may reflect an altered phenotype by EE. Moreover, there were large-sized correlations between percentage of neocortical and hippocampal microglia and cognitive outcomes, and these effects were moderated by genotype. The findings of this Chapter suggest that microglia play an important role in the association between EE and cognitive protection in the presence of Aβ neuropathology, however, further studies are required to clarify this association.

6.4 Integration of findings

The ageing of the global population is accompanied by an increasing and substantial social and financial impact of ageing-related diseases that cause dementia, for which there is currently no effective treatment or cure (Prince et al., 2015). There is a wealth of literature supporting early-life stimulation from the environment being beneficial in reducing dementia risk in later-life, however, there is limited research evidence for the related benefits of environmental stimulation through mid to late-life. Given that compelling evidence suggests that the adult brain has the capacity to undergo environment-induced plasticity, and Aβ production can be modulated by synaptic activity (Kamenetz et al., 2003), the overarching aim of this thesis was to investigate the effect of environmental stimulation from mid to later-life on healthy and pathological ageing.

Early-life EE applied to transgenic FAD models has been associated with cognitive improvement, and variable effects on amyloid pathology (Table 1). However, the effect of later-life interventions is not well understood. Moreover, while Aβ is a hallmark feature of AD, there is an array of molecular and cellular alterations characteristic of AD, and so previous studies in this domain that have focused on the effect of EE on cognitive function and amyloid pathology are potentially leaving underlying neural mechanisms on the positive effects of EE undetected.

This thesis has offered support for the concept that the ageing brain retains capacity for plasticity in non-pathological and pathological conditions. A consistent finding from both the mid and late-life EE paradigms was that EE was accompanied by an improvement in cognitive function. The finding of cognitive protection afforded by EE in early-life paradigms has been widely reported (Nithianantharajah & Hannan, 2006). However, whether EE has the potential to induce cognitive protection in the

ageing brain, has received limited attention. Considering the ageing of populations, and the societal demands placed by an increasing number of people experiencing cognitive deterioration, the findings presented in this thesis offer encouragement as to the efficacy of cognitive engagement therapies for the ageing population.

One contrasting finding of this thesis was that baseline assessment of short-term memory at 6 months of age by the Y maze revealed a deficit in APP/PS1 mice relative to Wts (Chapter 3). Despite this, baseline Y maze testing at 12 months of age did not show any genotype differences in performance levels (Chapter 5). This may have led to differential enrichment effects on cognitive outcome, as the APP/PS1 mice in the mid-life cohort had a memory deficit to be rescued, where the late-life cohort did not show a deficit relative to healthy Wt mice. This may have been due to the Wt mice developing an ageing-related decline to short-term memory function due to ageing at 12 months, and 'catching up' to the level of APP/PS1 mice. However, in both the mid and late-life cohort studies, EE exerted differential cognitive effects that were dependent on genotype, in which EE was associated with superior long-term memory function in healthy Wts, and superior short-term memory function in APP/PS1 mice. The research literature on cognitive decline in healthy ageing populations typically shows that short-memory remains relatively intact (Hedden & Gabrieli, 2004; Salthouse, 2011), whereas short-term memory impairment is an early cognitive symptom of AD (Weintraub et al., 2012). EE may therefore have targeted effects on regions, and subsequently functions, of vulnerability.

The positive effects on cognitive function demonstrated in both the mid-life and late-life studies were independent to alterations in Aβ load. Therefore, the findings of this thesis suggest that the cognitive benefits afforded by environmental stimulation from mid to late-life are not due to a reduction in Aβ neuropathology, as APP/PS1 mice

demonstrated a complete restoration of memory function to the level of healthy Wts, despite minimal modification of underlying pathology. These findings support the cognitive reserve theory, in which individuals who have lived a highly cognitively engaging life, are able to withstand a greater neuropathological burden while maintaining cognitive function (Stern, 2012). This finding is not in direct contradiction to the amyloid cascade hypothesis of AD, in which excess Aβ stimulates a pathological cascade leading to the cognitive dysfunction observed in AD (Hardy & Selkoe, 2002). This APP/PS1 mouse model possesses Aβ levels 300-500% over endogenous levels (Jankowsky et al., 2004), and memory impairment was observed in this model at 6-month baseline (Chapter 3). However, the FAD model exhibits a mild elevation in steady-state Aβ levels (Jankowsky et al., 2005), and the stimulation provided by EE appeared to overcome the impairment that may be induced by Aβ pathology, as observed in the SH condition across studies.

While no alterations to Aβ load were detected as a result of EE, the complex/novel paradigm (EE+) introduced in mid-life (Chapter 4), was associated with increased Aβ deposition in the hippocampus and increased Aβ production in the neocortex. The EE+ condition was also associated with increased stress-hormone levels in APP/PS1 mice, which appeared to drive the increases in amyloid. Together, these findings suggest that a mild form of stimulation from the environment leads to improved cognitive function in healthy and pathological ageing, independent of alterations in amyloid levels. However, in the case of existing AD-associated neuropathology, a more complex form of enrichment that involves exposure to novel situations, may increase levels of stress and exacerbate amyloid pathology.

While EE was not associated with a reduction in Aβ burden, the findings of this thesis involved the identification of the promotion of other factors, which potentially

mediate the cognitive protection provided by EE. The findings demonstrated an EE-induced increase in hippocampal BDNF (Chapter 3), which may offer a mechanism for improvements in short-term memory function in APP/PS1 EE-exposed mice in both mid-life and late-life groups. Compelling evidence indicated that BDNF exerts strong pro-survival effects on neurons under pathological conditions, in addition to promoting synaptic plasticity and connectivity (Lu et al., 2013). The APP/PS1 model also demonstrated an increase in synaptic density in the hippocampal CA1 subregion, a region particularly vulnerable to AD (Scheff et al., 2007), which was likely due to the increase in BDNF and associated increase in synaptogenesis. The EE-associated promotion of hippocampal BDNF provides a likely driving force underlying the increase in synaptic connectivity and restoration of short-term memory function in APP/PS1 mice, despite the increasing presence of Aβ pathology.

Microglia are also thought to support neuronal function by mediating neurotrophic factors, including BDNF, in the brain (Elkabes, DiCicco-Bloom, & Black, 1996). APP/PS1 mice were found to have an increase in microglia density in the hippocampus and neocortex relative to healthy ageing Wt mice; EE altered this effect, resulting in no genotype differences. This effect was hypothesised to reflect an alteration in phenotype, in which APP/PS1 mice from EE may exhibit a less reactive, pro-inflammatory microglial phenotype. This evidence together suggests that EE may promote microglial health, leading to increased expression of BDNF that results in heightened synaptic connectivity, and subsequent protection to hippocampal-dependent memory function. These findings suggest that stimulation from the environment can enhance the compensatory mechanisms that promote synaptic connectivity for existing pathology, allowing for a greater resilience of cognitive function.

6.5 Limitations

Much of the understanding that we have of the mechanisms that underlie the effects of experience on the human brain, comes from research conducted on animal models. In this regard, one central limitation of this thesis is the use of a transgenic mouse model of AD. This model possesses mutations in genes involved in familial cases of AD, however, familial AD is rare, and the majority of AD cases are sporadic (Sherrington et al., 1995; 1996). Moreover, while this transgenic model exhibits some cardinal features of the disease, no animal model fully recapitulates the entire disease process. For example, this APP/PS1 model does not develop one of the main pathological features of AD, neurofibrillary tangles (Garcia-Alloza et al., 2006). While the results of thesis should be interpreted with this limitation taken into account, the overarching aim of this thesis was to investigate the effects of enrichment on Aβ neuropathology and cognitive function. Healthy Wt mice do not develop AD sporadically as in human cases, and do not exhibit pathological features of the disease (McGowan, Eriksen, & Hutton, 2006). Therefore, this mouse model that does develop Aβ neuropathology and cognitive dysfunction (Jankowsky et al. 2004; Garcia-Alloza et al. 2006; Vickers et al. 2009) was an appropriate model. In addition, for this thesis, only male mice were used. This poses a limitation due to the results potentially only transferring to the male population. However, male mice were chosen in these studies, as there is an observed increase in Aβ burden in female mice; this association is not well understood (Wang et al., 2003), and could have potentially confounded the findings of this study.

It has been argued that many of the benefits of EE are due to enhanced physical activity, rather than cognitive stimulation (Pang & Hannan, 2013), which poses a major limitation to EE studies. EE involves a variety of sensory, cognitive, and physical

activity that cannot be separated in order to investigate the sole effects of a single modality. Intrinsically, it is not possible to reproduce the experience of education, leisure activity, and occupational attainment in an animal model, when aiming to recapitulate the building of cognitive reserve. While some parallels can be drawn between EE and cognitive lifestyle in humans, the stimulation provided by EE is not completely analogous to the lifetime of experience that contributes to cognitive reserve in people. However, even in humans, it is difficult to separate physical activity from cognitive stimulation, and other extraneous factors. Therefore, in both human and animal studies, it remains to be elucidated what effects cognitive stimulation has on the building of cognitive reserve. However, Cracchiolio et al. (2007) aimed to address what kind of activity conferred the most benefit in reducing dementia risk. It was found that "complete" housing encompassing physical, cognitive, and social stimulation, conferred the most cognitive benefit as compared to only social or physical exercise conditions.

Much of the data generated in this thesis showed moderate variability; it is plausible that some mice may have "engaged" more in the stimulating environment than others, which could potentially result in greater neural and functional gains. Future studies would benefit from recording activity in EE, which would allow the individual engagement of mice to be tracked. Subsequently, it could be determined whether individual patterns of activity may modulate the effects of enrichment on the brain and cognitive function. In addition, the efficacy of the EE paradigms on functional outcome was assessed by cognitive testing using the Y maze and the Barnes maze. Cognitive testing on mouse models is highly variable (Stewart et al., 2011), and the Y maze, while commonly used to assess spatial memory function (Webster et al., 2014), is not highly sensitive to subtle cognitive deficit (Stewart et al., 2011). Therefore, future research should use more sensitive measures, such as the Radial Arm Maze in detecting

cognitive dysfunction in animal models of early-stage disease (Stewart et al., 2011). Moreover, group housing of mice tends to generate a dominance hierarchy, which first, may have altered the level of engagement in EE. Second, EE has been associated with a loss in stability of the hierarchy (Haemisch, Voss, & Gärtner, 1994), which also could have potentially confounded findings.

While employing an EE paradigm with animal models poses inherent limitations, the effect of cognitive stimulation on the neural system could not have been investigated in a living human sample. Furthermore, an EE paradigm offers a controlled environment, allowing for genetic and environmental factors to be carefully controlled for. This is in direct contrast to human populations studies, in which there is an array of extraneous factors that are challenging to control for, potentially confounding findings.

6.6 Implications

The study of an EE paradigm in healthy and pathological ageing has allowed for some mechanistic insight into how stimulation from the environment might modulate the ageing nervous system and the neural response to AD pathogenesis. With further investigation, key molecular pathways stimulated by EE may be identified, allowing for the identification of therapeutic targets that could be promoted exogenously through a treatment strategy that parallels EE, or through the development of new drugs that could mimic the beneficial effects of EE, or 'enviromimetics' (Nithianantharajah & Hannan, 2006). In this thesis, an EE-associated increase in hippocampal BDNF in APP/PS1 mice was identified, which may have led to the increase in synaptic density and short-term memory function demonstrated in both mid and late-life cohorts. BDNF may therefore offer an encouraging molecular target for the promotion of cognitive health in healthy and pathological ageing.

The findings of this thesis demonstrate that the ageing neural system is capable of undergoing plastic alterations in response to stimulation from the environment, in both non-pathological and pathological conditions. Perhaps most remarkably, even in the presence of neuropathology, EE can induce compensatory mechanisms promoting synaptic connectivity and cognitive restoration. EE is a non-invasive therapy that confers powerful benefits, and has the potential to transfer into human interventions for pathological and non-pathological ageing.

The findings of this thesis also led to the identification of an abnormal stress-response in APP/PS1 mice that was associated with exacerbated pathology. This finding raises implications as to the importance of assessing forms of cognitive stimulation therapies for their intensiveness and potential to cause frustration and stress, particularly for people with existing dementia due to AD. Moreover, introducing stress-reduction strategies for people living with AD, may offer an efficacious strategy for slowing disease process progression.

6.7 Future research

Further studies would benefit from tying these findings together, to more fully elucidate the underlying mechanisms that mediate the effect of EE on cognitive function in models of ageing and AD-neuropathology. APP/PS1 mice demonstrated signs of a disruption to the balance between synaptic inhibition and excitation (Chapter 3). Therefore, it would be important to investigate microglia in the mid-life cohort. Activated microglia secrete interleukin-1β (IL-1β), with elevated levels in the hippocampus reported to be associated with cognitive impairment (Williamson et al., 2011), and with the disruption of the normal balance between synaptic excitation and inhibition (Patterson, 2015). Furthermore, one of the key mechanisms by which the brain regulates immune activity is via the HPA axis. Glucocorticoids can have opposing

effects on microglial activity (Ashwell, Lu, & Vacchio, 2000), which may have contributed to the exacerbation of Aβ detected in mice exposed to the EE+ condition (Chapter 4).

The findings presented in Chapter 4, of an elevated stress-response to complexity and novelty, and associated exacerbated pathology, has potential for a human translation study. Cognitive stimulation therapies have demonstrated variable effects on people living with dementia, and are often assumed to at least do no harm (Choi & Twamley, 2013). Despite this, the effects on stress-hormone levels in people living with dementia have not been reported. Future studies on cognitive interventions for people living with dementia should also analyse levels of stress, as this may hasten disease progression.

6.8 Conclusion

In conclusion, EE paradigms introduced through mid to later-life for both non-pathological and pathological ageing, are associated with some level of cognitive enhancement and/or resilience. The findings of this thesis suggest the protective effect of EE on cognitive function in a transgenic model of AD pathology, relies on an enhancement of synaptic connectivity and promotion of neural plasticity, as cognitive protection was observed with no evidence of an attenuation to existing Aβ neuropathology. EE is a non-invasive therapy, that confers many powerful benefits to the neural system in both ageing and pathological contexts. The EE paradigm has the potential to lead to a new approach to cognitive and/or behavioural therapies, or the development of new pharmacological treatments, for the treatment of ageing-associated cognitive decline and dementia.

Chapter 7

References

Aliaga, E., Silhol, M., Bonneau, N., Maurice, T., Arancibia, S., & Tapia-Arancibia, L. (2010). Dual response of BDNF to sublethal concentrations of beta-amyloid peptides in cultured cortical neurons. *Neurobiology of Disease*, *37*(1), 208–17. http://doi.org/10.1016/j.nbd.2009.10.004

Anderson, M. I., Killing, S., Morris, C., O'Donoghue, A., Onyiagha, D., Stevenson, R., … Jeffery, K. J. (2006). Behavioral correlates of the distributed coding of spatial context. *Hippocampus*, *16*(9), 730–742. http://doi.org/10.1002/hipo.20206

Anstey, K., & Christensen, H. (2000). Education, activity, health, blood pressure and apolipoprotein E as predictors of cognitive change in old age: a review. *Gerontology*, *46*(3), 163–177. http://doi.org/10.1159/000022153

Arancibia, S., Silhol, M., Moulière, F., Meffre, J., Höllinger, I., Maurice, T., & Tapia-Arancibia, L. (2008). Protective effect of BDNF against beta-amyloid induced neurotoxicity in vitro and in vivo in rats. *Neurobiology of Disease*, *31*(3), 316–326. http://doi.org/10.1016/j.nbd.2008.05.012

Ard, M. D., Cole, G. M., Wei, J., Mehrle, P., & Fratkin, J. D. (1996). Scavenging of Alzheimer's amyloid beta-protein by microglia in culture. *Journal of Neuroscience Research*, *43*(2), 190–202. http://doi.org/10.1002/(SICI)1097-4547(19960115)43:2<190::AID-JNR7>3.0.CO;2-B

Arellano, J. I., Guadiana, S. M., Breunig, J. J., Rakic, P., & Sarkisian, M. R. (2012). Development and distribution of neuronal cilia in mouse neocortex. *Journal of Comparative Neurology*, *520*(4), 848–873. http://doi.org/10.1002/cne.22793

Arendash, G. W., Garcia, M. F., Costa, D. A., Cracchiolo, J. R., Wefes, I. M., & Potter, H. (2004). Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable beta-amyloid deposition. *Neuroreport*, *15*(11),

1751–1754. http://doi.org/10.1097/01.wnr.0000137183.68847

Arendt, T. (2009). Synaptic degeneration in Alzheimer's disease. *Acta Neuropathologica*. http://doi.org/10.1007/s00401-009-0536-x

Arriagada, P. V, Growdon, J. H., Hedleywhyte, E. T., & Hyman, B. T. (1992). Neurofibrillary Tangles But Not Senile Plaques Parallel Duration and Severity of Alzheimers-Disease. *Neurology*, *42*(3), 631–639. http://doi.org/10.1212/WNL.42.3.631

Ashwell, J. D., Lu, F. W., & Vacchio, M. S. (2000). Glucocorticoids in T cell development and function. *Annual Review of Immunology*, *18*(18), 309–45. http://doi.org/10.1146/annurev.immunol.18.1.309

Aso, E., Lomoio, S., López-González, I., Joda, L., Carmona, M., Fernández-Yagüe, N., … Ferrer, I. (2012). Amyloid generation and dysfunctional immunoproteasome activation with disease progression in animal model of familial Alzheimer's disease. *Brain Pathology*, *22*(5), 636–653. http://doi.org/10.1111/j.1750-3639.2011.00560.x

Baglietto-Vargas, D., Chen, Y., Suh, D., Ager, R. R., Rodriguez-Ortiz, C. J., Medeiros, R., … LaFerla, F. M. (2015). Short-term modern life-like stress exacerbates Aβ pathology and synapse loss in 3xTg-AD mice. *Journal of Neurochemistry*, *134*(5), 915–926. http://doi.org/10.1111/jnc.13195

Barde, Y. A., Edgar, D., & Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *The EMBO Journal*, *1*(5), 549–53. http://doi.org/0261-4189/82/0105-0549

Barnes, D., & Yaffe, K. (2013). The Projected Impact of Risk Factor Reduction on Alzheimer's Disease Prevalence. *Lancet Neurology*, *10*(9), 819–828. http://doi.org/10.1016/S1474-4422(11)70072-2.The

Bennett, D. a, Wilson, R. S., Schneider, J. a, Evans, D. a, Mendes de Leon, C. F., Arnold, S. E., … Bienias, J. L. (2003). Education modifies the relation of AD pathology to level of cognitive function in older persons. *Neurology*, *60*, 1909–1915. http://doi.org/10.1212/01.WNL.0000069923.64550.9F

Berg, L., McKeel, D. W., Miller, J. P., Storandt, M., Rubin, E. H., Morris, J. C., … Saunders, A. M. (1998). Clinicopathologic Studies in Cognitively Healthy Aging and Alzheimer Disease. *Archives of Neurology*, *55*(3), 326. http://doi.org/10.1001/archneur.55.3.326

Besser, S., Sicker, M., Marx, G., Winkler, U., Eulenburg, V., Hülsmann, S., & Hirrlinger, J. (2015). A transgenic mouse line expressing the red fluorescent protein tdTomato in GABAergic neurons. *PLoS ONE*, *10*(6). http://doi.org/10.1371/journal.pone.0129934

Birch, A. M., Mcgarry, N. B., & Kelly, Á. M. (2013). Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. *Hippocampus*, *23*(6), 437–450. http://doi.org/10.1002/hipo.22103

Bolmont, T., Haiss, F., Eicke, D., Radde, R., Mathis, C. A., Klunk, W. E., … Calhoun, M. E. (2008). Dynamics of the microglial/amyloid interaction indicate a role in plaque maintenance. *J Neurosci*, *28*(16), 4283–4292. http://doi.org/10.1523/JNEUROSCI.4814-07.2008

Boncristiano, S., Calhoun, M. E., Howard, V., Bondolfi, L., Kaeser, S. A., Wiederhold, K. H., … Jucker, M. (2005). Neocortical synaptic bouton number is maintained despite robust amyloid deposition in APP23 transgenic mice. *Neurobiology of Aging*, *26*(5), 607–613. http://doi.org/10.1016/j.neurobiolaging.2004.06.010

Braak, H., & Del Tredici, K. (2011). The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathologica*, *121*(2), 171–181. http://doi.org/10.1007/s00401-010-0789-4

Bradshaw, E. M., Chibnik, L. B., Keenan, B. T., Ottoboni, L., Raj, T., Tang, A., … De Jager, P. L. (2013). CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nature Neuroscience*, *16*(7), 848–850. http://doi.org/10.1038/nn.3435

Cabelli, R. J., Hohn, A., & Shatz, C. J. (1995). Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. *Science (New York, N.Y.)*, *267*(5204), 1662–6. http://doi.org/10.1126/science.7886458

Carpenter, A. F., Carpenter, P. W., & Markesbery, W. R. (1993). Morphometric analysis of microglia in Alzheimer's disease. *Journal of Neuropathology and Experimental Neurology*, *52*(6), 601–8. http://doi.org/10.1097/00005072-199311000-00007

Carroll, J. C., Iba, M., Bangasser, D. A., Valentino, R. J., James, M. J., Brunden, K. R., … Trojanowski, J. Q. (2011). Chronic Stress Exacerbates Tau Pathology , Neurodegeneration , and Cognitive Performance through a Mechanism in a Transgenic Mouse Model of Tauopathy. *Journal of Neuroscience*, *31*(40), 14436–14449. http://doi.org/10.1523/JNEUROSCI.3836-11.2011

Catlow, B. J., Rowe, A. R., Clearwater, C. R., Mamcarz, M., Arendash, G. W., & Sanchez-Ramos, J. (2009). Effects of environmental enrichment and physical activity on neurogenesis in transgenic PS1/APP mice. *Brain Research*, *1256*, 173–179. http://doi.org/10.1016/j.brainres.2008.12.028

Chapman, P. F., White, G. L., Jones, M. W., Cooper-Blacketer, D., Marshall, V. J., Irizarry, M., … Hsiao, K. K. (1999). Impaired synaptic plasticity and learning in

aged amyloid precursor protein transgenic mice. *Nature Neuroscience*, *2*(3), 271–276. http://doi.org/10.1038/6374

Chen, Z., Jalabi, W., Hu, W., Park, H.-J., Gale, J. T., Kidd, G. J., … Trapp, B. D. (2014). Microglial displacement of inhibitory synapses provides neuroprotection in the adult brain. *Nature Communications*, *5*(July), 4486. http://doi.org/10.1038/ncomms5486

Choi, J., & Twamley, E. W. (2013). Cognitive rehabilitation therapies for Alzheimer's disease: A review of methods to improve treatment engagement and self-efficacy. *Neuropsychology Review*. http://doi.org/10.1007/s11065-013-9227-4

Choi, S. H., Veeraraghavalu, K., Lazarov, O., Marler, S., Ransohoff, R. M., Ramirez, J. M., & Sisodia, S. S. (2008). Non-Cell-Autonomous Effects of Presenilin 1 Variants on Enrichment-Mediated Hippocampal Progenitor Cell Proliferation and Differentiation. *Neuron*, *59*(4), 568–580. http://doi.org/10.1016/j.neuron.2008.07.033

Cohen-Cory, S., & Fraser, S. E. (1995). Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. *Nature*. http://doi.org/10.1038/378192a0

Coleman, P., Federoff, H., & Kurlan, R. (2004). A focus on the synapse for neuroprotection in Alzheimer disease and other dementias. *Neurology*, *63*(7), 1155–62. http://doi.org/10.1212/01.WNL.0000140626.48118.0A

Collins, J. M., King, A. E., Woodhouse, A., Kirkcaldie, M. T. K., & Vickers, J. C. (2015). The effect of focal brain injury on beta-amyloid plaque deposition, inflammation and synapses in the APP/PS1 mouse model of Alzheimer's disease. *Experimental Neurology*, *267*, 219–29. http://doi.org/10.1016/j.expneurol.2015.02.034

Combs, C. K., Johnson, D. E., Cannady, S. B., Lehman, T. M., & Landreth, G. E. (1999). Identification of microglial signal transduction pathways mediating a neurotoxic response to amyloidogenic fragments of beta-amyloid and prion proteins. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(3), 928–39. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9920656

Combs, C. K., Johnson, D. E., Karlo, J. C., Cannady, S. B., & Landreth, G. E. (2000). Inflammatory mechanisms in Alzheimer's disease: inhibition of beta-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(2), 558–67. Retrieved from http://www.jneurosci.org/content/20/2/558.abstract

Condello, C., Yuan, P., Schain, A., & Grutzendler, J. (2015). Microglia constitute a barrier that prevents neurotoxic protofibrillar Aβ42 hotspots around plaques. *Nature Communications*, *6*(May 2014), 6176. http://doi.org/10.1038/ncomms7176

Connor, J. R., Wang, E. C., & Diamond, M. C. (1982). Increased length of terminal dendritic segments in old adult rats' somatosensory cortex: An environmentally induced response. *Experimental Neurology*, *78*(2), 466–470. http://doi.org/10.1016/0014-4886(82)90064-4

Costa, D. a., Cracchiolo, J. R., Bachstetter, A. D., Hughes, T. F., Bales, K. R., Paul, S. M., … Potter, H. (2007). Enrichment improves cognition in AD mice by amyloid-related and unrelated mechanisms. *Neurobiology of Aging*, *28*, 831–844. http://doi.org/10.1016/j.neurobiolaging.2006.04.009

Cotel, M.-C., Jawhar, S., Christensen, D. Z., Bayer, T. a, & Wirths, O. (2012). Environmental enrichment fails to rescue working memory deficits, neuron loss,

and neurogenesis in APP/PS1KI mice. *Neurobiology of Aging*, *33*(1), 96–107. http://doi.org/10.1016/j.neurobiolaging.2010.02.012

Cowansage, K. K., LeDoux, J. E., & Monfils, M. H. (2010). Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity. *Curr Mol Pharmacol*, *3*(1), 12–29. http://doi.org/EPub-Abstract-CMP-03 [pii]

Cracchiolo, J. R., Mori, T., Nazian, S. J., Tan, J., Potter, H., & Arendash, G. W. (2007). Enhanced cognitive activity-over and above social or physical activity-is required to protect Alzheimer's mice against cognitive impairment, reduce A?? deposition, and increase synaptic immunoreactivity. *Neurobiology of Learning and Memory*, *88*(3), 277–294. http://doi.org/10.1016/j.nlm.2007.07.007

Crofton, E. J., Zhang, Y., & Green, T. A. (2015). Inoculation stress hypothesis of environmental enrichment. *Neuroscience and Biobehavioral Reviews*. http://doi.org/10.1016/j.neubiorev.2014.11.017

Csernansky, J. G., Dong, H., Ph, D., Fagan, A. M., Wang, L., Xiong, C., … Morris, J. C. (2006). Plasma Cortisol and Progression of Dementia in DAT Subjects. *American Journal of Psychiatry*, *163*(December), 2164–2169. http://doi.org/10.1176/appi.ajp.163.12.2164

Csicsvari, J., O'Neill, J., Allen, K., & Senior, T. (2007). Place-selective firing contributes to the reverse-order reactivation of CA1 pyramidal cells during sharp waves in open-field exploration. *European Journal of Neuroscience*, *26*(3), 704–716. http://doi.org/10.1111/j.1460-9568.2007.05684.x

DeKosky, S. T., & Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Annals of Neurology*, *27*(5), 457–464. http://doi.org/10.1002/ana.410270502

Dellu, F., Contarino, a, Simon, H., Koob, G. F., & Gold, L. H. (2000). Genetic

differences in response to novelty and spatial memory using a two-trial recognition task in mice. *Neurobiology of Learning and Memory*, *73*(1), 31–48. http://doi.org/10.1006/nlme.1999.3919

Derecki, N. C., Katzmarski, N., Kipnis, J., & Meyer-Luehmann, M. (2014). Microglia as a critical player in both developmental and late-life CNS pathologies. *Acta Neuropathologica*. http://doi.org/10.1007/s00401-014-1321-z

Dickson, T. C., & Vickers, J. C. (2001). The morphological phenotype of β-amyloid plaques and associated neuritic changes in Alzheimer's disease. *Neuroscience*, *105*(1), 99–107. http://doi.org/10.1016/S0306-4522(01)00169-5

Dong, H., Goico, B., Martin, M., Csernansky, C. A., Bertchume, A., & Csernansky, J. G. (2004). Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience*, *127*(3), 601–609. http://doi.org/10.1016/j.neuroscience.2004.05.040

Dong, H., Martin, M. V., Chambers, S., & Csernansky, J. G. (2007). Spatial relationship between synapse loss and β-amyloid deposition in Tg2576 mice. *Journal of Comparative Neurology*, *500*(2), 311–321. http://doi.org/10.1002/cne.21176

Ehninger, D., & Kempermann, G. (2003). Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. *Cerebral Cortex*, *13*(8), 845–851. http://doi.org/10.1093/cercor/13.8.845

Elkabes, S., DiCicco-Bloom, E. M., & Black, I. B. (1996). Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(8), 2508–2521.

http://doi.org/10.1017/CBO9781107415324.004

Erickson, K.I., Miller, D.L., & Roecklein, K.A. (2012). The aging hippocampus: interactions between exercise, depression, and BDNF. *Neuroscientist, 18,* (1): 82-97. doi: 10.1177/1073858410397054

Fazzari, P., Snellinx, A., Sabanov, V., Ahmed, T., Serneels, L., Gartner, A., … De Strooper, B. (2014). Cell autonomous regulation of hippocampal circuitry via Aph1b-γ-secretase/neuregulin 1 signalling. *eLife*, *2014*(3). http://doi.org/10.7554/eLife.02196.001

Ferrer, I., Marín, C., Rey, M. J., Ribalta, T., Goutan, E., Blanco, R., … Martí, E. (1999). BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *Journal of Neuropathology and Experimental Neurology*, *58*(7), 729–39. http://doi.org/10.1097/00005072-199907000-00007

Flanary, B. E., Sammons, N. W., Nguyen, C., Walker, D., & Streit, W. J. (2007). Evidence that aging and amyloid promote microglial cell senescence. *Rejuvenation Research*, *10*(1), 61–74. http://doi.org/10.1089/rej.2006.9096

Floden, A. M., & Combs, C. K. (2011). Microglia demonstrate age-dependent interaction with amyloid-β fibrils. *Journal of Alzheimer's Disease*, *25*(2), 279–293. http://doi.org/10.3233/JAD-2011-101014

Flores-Otero, J., & Davis, R. L. (2011). Synaptic proteins are tonotopically graded in postnatal and adult type I and type II spiral ganglion neurons. *Journal of Comparative Neurology*, *519*(8), 1455–1475. http://doi.org/10.1002/cne.22576

Fong, A. Y., Stornetta, R. L., Foley, C. M., & Potts, J. T. (2005). Immunohistochemical localization of GAD67-expressing neurons and processes in the rat brainstem:

Subregional distribution in the nucleus tractus solitarius. *Journal of Comparative Neurology*, *493*(2), 274–290. http://doi.org/10.1002/cne.20758

Frick, K. M., & Fernandez, S. M. (2003). Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. *Neurobiology of Aging*, *24*(4), 615–626. http://doi.org/10.1016/S0197-4580(02)00138-0

Friedland, R. P., Fritsch, T., Smyth, K. A., Koss, E., Lerner, A. J., Chen, C. H., … Debanne, S. M. (2001). Patients with Alzheimer's disease have reduced activities in midlife compared with healthy control-group members. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(6), 3440–5. http://doi.org/10.1073/pnas.061002998

Fu, W., Lu, C., & Mattson, M. P. (2002). Telomerase Mediates the Cell Survival-Promoting Actions of Brain-Derived Neurotrophic Factor and Secreted Amyloid Precursor Protein in Developing Hippocampal Neurons. *J. Neurosci.*, *22*(24), 10710–10719. http://doi.org/22/24/10710 [pii]

Fuchs, E., & Flügge, G. (2014). Adult neuroplasticity: More than 40 years of research. *Neural Plasticity*. http://doi.org/10.1155/2014/541870

Garcia-Alloza, M., Robbins, E. M., Zhang-Nunes, S. X., Purcell, S. M., Betensky, R. A., Raju, S., … Frosch, M. P. (2006). Characterization of amyloid deposition in the APPs... [Neurobiol Dis. 2006] - PubMed result. *Neurobiology of Disease*, *24*(3), 516–24. http://doi.org/10.1016/j.nbd.2006.08.017

Gelfo, F., De Bartolo, P., Giovine, A., Petrosini, L., & Leggio, M. G. (2009). Layer and regional effects of environmental enrichment on the pyramidal neuron morphology of the rat. *Neurobiology of Learning and Memory*, *91*(4), 353–365. http://doi.org/10.1016/j.nlm.2009.01.010

Giannakopoulos, P., Herrmann, F. R., Bussière, T., Bouras, C., Kövari, E., Perl, D. P.,

… Hof, P. R. (2003). Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology*, *60*(9), 1495–500. http://doi.org/10.1212/01.WNL.0000063311.58879.01

Gonzá lez-Colaço Harmand, M., Meillon, C., Rullier, L., Avila-Funes, J. A., Bergua, V., Dartigues, J. F., & Amieva, H. (2014). Cognitive decline after entering a nursing home: A 22-year follow-up study of institutionalized and noninstitutionalized elderly people. *Journal of the American Medical Directors Association*, *15*(7), 504–508. http://doi.org/10.1016/j.jamda.2014.02.006

Green, K. N., Billings, L. M., Roozendaal, B., McGaugh, J. L., & LaFerla, F. M. (2006). Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(35), 9047–9056. http://doi.org/10.1523/JNEUROSCI.2797-06.2006

Greenough, W. T., Volkmar, F. R., & Juraska, J. M. (1973). Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. *Experimental Neurology*, *41*(2), 371–378. http://doi.org/10.1016/0014-4886(73)90278-1

Griciuc, A., Serrano-Pozo, A., Parrado, A. R., Lesinski, A. N., Asselin, C. N., Mullin, K., … Tanzi, R. E. (2013). Alzheimer's disease risk gene cd33 inhibits microglial uptake of amyloid beta. *Neuron*, *78*(4), 631–643. http://doi.org/10.1016/j.neuron.2013.04.014

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., … Alzheimer Genetic Analysis Group. (2013). TREM2 variants in Alzheimer's disease. *The New England Journal of Medicine*, *368*(2), 117–27. http://doi.org/10.1056/NEJMoa1211851

Guo, Q., Zheng, H., & Justice, N. J. (2012). Central CRF system perturbation in an Alzheimer's disease knockin mouse model. *Neurobiology of Aging*, *33*(11), 2678–2691. http://doi.org/10.1016/j.neurobiolaging.2012.01.002

Haemisch, A., Voss, T., & Gärtner, K. (1994). Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. *Physiology and Behavior*, *56*(5), 1041–1048. http://doi.org/10.1016/0031-9384(94)90341-7

Hardy, J. (1997). The Alzheimer family of diseases: many etiologies, one pathogenesis? *Proceedings of the National Academy of Sciences of the United States of America*, *94*(6), 2095–2097. http://doi.org/10.1073/pnas.94.6.2095

Hardy, J., & Selkoe, D. J. (2002a). Amyloid and Hypothesis of Problems Alzheimer's Road to Disease : the Therapeutics. *Science*, *297*(5580), 353–356.

Hardy, J., & Selkoe, D. J. (2002b). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science (New York, N.Y.)*, *297*(5580), 353–356. http://doi.org/10.1126/science.1072994

Harvey, K., Duguid, I. C., Alldred, M. J., Beatty, S. E., Ward, H., Keep, N. H., … Harvey, R. J. (2004). The GDP-GTP exchange factor collybistin: an essential determinant of neuronal gephyrin clustering. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(25), 5816–5826. http://doi.org/10.1523/JNEUROSCI.1184-04.2004

Hedden, T., & Gabrieli, J. D. E. (2004). Insights into the ageing mind: a view from cognitive neuroscience. *Nature Reviews. Neuroscience*, *5*(2), 87–96. http://doi.org/10.1038/nrn1323

Hedden, T., Oh, H., Younger, A. P., & Patel, T. A. (2013). Meta-analysis of amyloid-cognition relations in cognitively normal older adults. *Neurology*, *80*(14), 1341–

1348. http://doi.org/10.1212/WNL.0b013e31828ab35d

Heneka, M. T., Carson, M. J., Khoury, J. El, Landreth, G. E., Brosseron, F., Feinstein, D. L., … Kummer, M. P. (2015). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*. http://doi.org/10.1016/S1474-4422(15)70016-5

Herman, J. P., Schäfer, M. K., Young, E. a, Thompson, R., Douglass, J., Akil, H., & Watson, S. J. (1989). Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *9*(9), 3072–82. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2795152

Herring, A., Lewejohann, L., Panzer, A.-L., Donath, A., Kröll, O., Sachser, N., … Keyvani, K. (2011). Preventive and therapeutic types of environmental enrichment counteract beta amyloid pathology by different molecular mechanisms. *Neurobiology of Disease*, *42*(3), 530–8. http://doi.org/10.1016/j.nbd.2011.03.007

Heurtaux, T., Michelucci, A., Losciuto, S., Gallotti, C., Felten, P., Dorban, G., … Heuschling, P. (2010). Microglial activation depends on beta-amyloid conformation: Role of the formylpeptide receptor 2. *Journal of Neurochemistry*, *114*(2), 576–586. http://doi.org/10.1111/j.1471-4159.2010.06783.x

Hickman, S. E., Allison, E. K., & El Khoury, J. (2008). Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(33), 8354–60. http://doi.org/10.1523/JNEUROSCI.0616-08.2008

Hock, C., Heese, K., Hulette, C., Rosenberg, C., & Otten, U. (2000). Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Archives of Neurology*, *57*(6), 846–851.

http://doi.org/10.1001/archneur.57.6.846

Hofer, S. M., Berg, S., & Era, P. (2003). Evaluating the interdependence of aging-related changes in visual and auditory acuity, balance, and cognitive functioning. *Psychology and Aging*, *18*(2), 285–305. http://doi.org/10.1037/0882-7974.18.2.285

Holmes, C., Cunningham, C., Zotova, E., Woolford, J., Dean, C., Kerr, S., … Perry, V. H. (2009). Systemic inflammation and disease progression in Alzheimer disease. *Neurology*, *73*(10), 768–774. http://doi.org/10.1212/WNL.0b013e3181b6bb95

Holmes, S., Kapelner, A., & Lee, P. P. (2009). An Interactive Java Statistical Image Segmentation System: GemIdent. *Journal of Statistical Software*, *30*(10).

Hu, L., Wong, T. P., Côté, S. L., Bell, K. F. S., & Cuello, A. C. (2003). The impact of Aβ-plaques on cortical cholinergic and non-cholinergic presynaptic boutons in alzheimer's disease-like transgenic mice. *Neuroscience*, *121*(2), 421–432. http://doi.org/10.1016/S0306-4522(03)00394-4

Ickes, B. R., Pham, T. M., Sanders, L. A., Albeck, D. S., Mohammed, A. H., & Granholm, A.-C. (2000). Long-Term Environmental Enrichment Leads to Regional Increases in Neurotrophin Levels in Rat Brain. *Experimental Neurology*, *164*(1), 45–52. http://doi.org/10.1006/exnr.2000.7415

Isaacson, J. S., & Scanziani, M. (2011). How inhibition shapes cortical activity. *Neuron*. http://doi.org/10.1016/j.neuron.2011.09.027

Itagaki, S., McGeer, P. L., Akiyama, H., Zhu, S., & Selkoe, D. (1989). Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *Journal of Neuroimmunology*, *24*(3), 173–182. http://doi.org/10.1016/0165-5728(89)90115-X

Ito, D., Imai, Y., Ohsawa, K., Nakajima, K., Fukuuchi, Y., & Kohsaka, S. (1998). Microglia-specific localisation of a novel calcium binding protein, Iba1. *Molecular Brain Research*, *57*(1), 1–9. http://doi.org/10.1016/S0169-328X(98)00040-0

Izco, M., Martínez, P., Corrales, A., Fandos, N., García, S., Insua, D., … Sarasa, M. (2014). Changes in the brain and plasma Aβ peptide levels with age and its relationship with cognitive impairment in the APPswe/PS1dE9 mouse model of Alzheimer's disease. *Neuroscience*, *263*, 269–279. http://doi.org/10.1016/j.neuroscience.2014.01.003

Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocrine Reviews*. http://doi.org/10.1210/edrv-12-2-118

Jagust, W. (2016). Is amyloid-β harmful to the brain? Insights from human imaging studies. *Brain*, *139*(1), 23–30. http://doi.org/10.1093/brain/awv326

Jankowsky, J. L., Melnikova, T., Fadale, D. J., Xu, G. M., Slunt, H. H., Gonzales, V., … Savonenko, A. V. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *25*(21), 5217–24. http://doi.org/10.1523/JNEUROSCI.5080-04.2005

Jankowsky, J. L., Slunt, H. H., Gonzales, V., Jenkins, N. A., Copeland, N. G., & Borchelt, D. R. (2004). APP processing and amyloid deposition in mice haplo-insufficient for presenilin 1. *Neurobiology of Aging*, *25*(7), 885–92. http://doi.org/10.1016/j.neurobiolaging.2003.09.008

Jankowsky, J. L., Xu, G., Fromholt, D., Gonzales, V., & Borchelt, D. R. (2003). Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. *Journal of Neuropathology and Experimental Neurology*, *62*(12), 1220–1227.

Jansen, D., Janssen, C. I. F., Vanmierlo, T., Dederen, P. J., Van Rooij, D., Zinnhardt, B., … Kiliaan, A. J. (2012). Cholesterol and synaptic compensatory mechanisms

in Alzheimer's disease mice brain during aging. *Journal of Alzheimer's Disease*, *31*(4), 813–826. http://doi.org/10.3233/JAD-2012-120298

Jellinger, K. A., & Attems, J. (2013). Neuropathological approaches to cerebral aging and neuroplasticity. *Dialogues in Clinical Neuroscience*, *15*(1), 29–43.

Jeong, Y. H., Kim, J. M., Yoo, J., Lee, S. H., Kim, H. S., & Suh, Y. H. (2011). Environmental enrichment compensates for the effects of stress on disease progression in Tg2576 mice, an Alzheimer's disease model. *Journal of Neurochemistry*, *119*(6), 1282–1293. http://doi.org/10.1111/j.1471-4159.2011.07514.x

Jeong, Y. H., Park, C. H., Yoo, J., Shin, K. Y., Ahn, S.-M., Kim, H.-S., … Suh, Y.-H. (2006). Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *20*, 729–731. http://doi.org/10.1096/fj.05-4265fje

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P. V, Snaedal, J., … Stefansson, K. (2013). Variant of TREM2 associated with the risk of Alzheimer's disease. *N Engl J Med*, *368*(2), 107–116. http://doi.org/10.1056/NEJMoa1211103

Jung, C. K. E., & Herms, J. (2014). Structural dynamics of dendritic spines are influenced by an environmental enrichment: an in vivo imaging study. *Cerebral Cortex (New York, N.Y. : 1991)*, *24*(2), 377–84. http://doi.org/10.1093/cercor/bhs317

Kai, H., Shin, R.-W., Ogino, K., Hatsuta, H., Murayama, S., & Kitamoto, T. (2012). Enhanced antigen retrieval of amyloid β immunohistochemistry: re-evaluation of amyloid β pathology in Alzheimer disease and its mouse model. *The Journal of*

Histochemistry and Cytochemistry : Official Journal of the Histochemistry Society, *60*(10), 761–9. http://doi.org/10.1369/0022155412456379

Kamegaya, T., Araki, Y., Kigure, H., & Yamaguchi, H. (2014). Twelve-week physical and leisure activity programme improved cognitive function in community-dwelling elderly subjects: A randomized controlled trial. *Psychogeriatrics*, *14*(1), 47–54. http://doi.org/10.1111/psyg.12038

Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., … Malinow, R. (2003). APP Processing and Synaptic Function. *Neuron*, *37*(6), 925–937. http://doi.org/10.1016/S0896-6273(03)00124-7

Karlsson, M. P., & Frank, L. M. (2008). Network dynamics underlying the formation of sparse, informative representations in the hippocampus. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *28*(52), 14271–14281. http://doi.org/10.1523/JNEUROSCI.4261-08.2008

Katzman, R., Aronson, M., Fuld, P., Kawas, C., Brown, T., Morgenstern, H., … Ooi, W. L. (1989). Development of dementing illnesses in an 80-year-old volunteer cohort. *Ann Neurol*, *25*, 317–324. http://doi.org/10.1002/ana.410250402

Katzman, R., Terry, R., DeTeresa, R., Brown, T., Davies, P., Fuld, P., … Peck, a. (1988). Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Annals of Neurology*, *23*(2), 138–144. http://doi.org/10.1002/ana.410230206

Ke, H. C., Huang, H. J., Liang, K. C., & Hsieh-Li, H. M. (2011). Selective improvement of cognitive function in adult and aged APP/PS1 transgenic mice by continuous non-shock treadmill exercise. *Brain Research*, *1403*, 1–11. http://doi.org/10.1016/j.brainres.2011.05.056

Kealy, J., & Commins, S. (2010). Frequency-dependent changes in synaptic plasticity

and brain-derived neurotrophic factor (BDNF) expression in the CA1 to perirhinal cortex projection. *Brain Research*, *1326*, 51–61. http://doi.org/10.1016/j.brainres.2010.02.065

Kesner, R. P. (2007). Behavioral functions of the CA3 subregion of the hippocampus. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *14*(11), 771–781. http://doi.org/10.1101/lm.688207

King, D. L., & Arendash, G. W. (2002). Maintained synaptophysin immunoreactivity in Tg2576 transgenic mice during aging: Correlations with cognitive impairment. *Brain Research*, *926*(1–2), 58–68. http://doi.org/10.1016/S0006-8993(01)03294-2

Kolb, B., Gibb, R., & Gorny, G. (2003). Experience-dependent changes in dendritic arbor and spine density in neocortex vary qualitatively with age and sex. *Neurobiology of Learning and Memory*, *79*(1), 1–10. http://doi.org/10.1016/S1074-7427(02)00021-7

Krabbe, G., Halle, A., Matyash, V., Rinnenthal, J. L., Eom, G. D., Bernhardt, U., … Heppner, F. L. (2013). Functional Impairment of Microglia Coincides with Beta-Amyloid Deposition in Mice with Alzheimer-Like Pathology. *PLoS ONE*, *8*(4). http://doi.org/10.1371/journal.pone.0060921

Kuzumaki, N., Ikegami, D., Tamura, R., Hareyama, N., Imai, S., Narita, M., … Narita, M. (2011). Hippocampal epigenetic modification at the brain-derived neurotrophic factor gene induced by an enriched environment. *Hippocampus*, *21*(2), 127–132. http://doi.org/10.1002/hipo.20775

Lambert, T. J., Fernandez, S. M., & Frick, K. M. (2005). Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiol Learn Mem*, *83*(3), 206–216. http://doi.org/10.1016/j.nlm.2004.12.001

Lazarov, O., Robinson, J., Tang, Y. P., Hairston, I. S., Korade-Mirnics, Z., Lee, V. M. Y., … Sisodia, S. S. (2005). Environmental enrichment reduces Aβ levels and amyloid deposition in transgenic mice. *Cell*, *120*(5), 701–713. http://doi.org/10.1016/j.cell.2005.01.015

Lee, C. Y. D., & Landreth, G. E. (2010). The role of microglia in amyloid clearance from the AD brain. *Journal of Neural Transmission*. http://doi.org/10.1007/s00702-010-0433-4

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research*, *163*(1), 78–90. http://doi.org/10.1016/j.bbr.2005.04.009

Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., & Vickers, J. C. (2014). Relationship between education and age-related cognitive decline: a review of recent research. *Psychogeriatrics : The Official Journal of the Japanese Psychogeriatric Society*. http://doi.org/10.1111/psyg.12083

Lenehan, M. E., Summers, M. J., Saunders, N. L., Summers, J. J., Ward, D. D., Ritchie, K., … Lenehan, C. (2015). Sending Your Grandparents to University Increases Cognitive Reserve: The Tasmanian Healthy Brain Project. *Neuropsychology*. http://doi.org/10.1037/neu0000249

Levi, O., Jongen-Relo, A. L., Feldon, J., Roses, A. D., & Michaelson, D. M. (2003). ApoE4 impairs hippocampal plasticity isoform-specifically and blocks the environmental stimulation of synaptogenesis and memory. *Neurobiology of Disease*, *13*(3), 273–282. http://doi.org/10.1016/S0969-9961(03)00045-7

Li, Y., Hu, J., Höfer, K., Wong, A. M. S., Cooper, J. D., Birnbaum, S. G., … Hofmann, S. L. (2010). DHHC5 interacts with PDZ domain 3 of post-synaptic density-95

(PSD-95) protein and plays a role in learning and memory. *Journal of Biological Chemistry*, *285*(17), 13022–13031. http://doi.org/10.1074/jbc.M109.079426

Lu, B. (2003). BDNF and Activity-Dependent Synaptic Modulation. *Learning and Memory*, *10*(2), 86–98. http://doi.org/10.1101/lm.54603.and

Lu, B., Nagappan, G., Guan, X., Nathan, P. J., & Wren, P. (2013). BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nature Reviews Neuroscience*, *14*(6), 1–16. http://doi.org/10.1038/nrn3505

Ma, T., & Klann, E. (2012). Amyloid-β: linking synaptic plasticity failure to memory disruption in Alzheimer's disease. *Journal of Neurochemistry*. http://doi.org/10.1111/j.1471-4159.2011.07506.x

Majumdar, A., Capetillo-Zarate, E., Cruz, D., Gouras, G. K., & Maxfield, F. R. (2011). Degradation of Alzheimer's amyloid fibrils by microglia requires delivery of ClC-7 to lysosomes. *Molecular Biology of the Cell*, *22*(10), 1664–76. http://doi.org/10.1091/mbc.E10-09-0745

Malik, R., & Chattarji, S. (2012). Enhanced intrinsic excitability and EPSP-spike coupling accompany enriched environment-induced facilitation of LTP in hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology*, *107*(5), 1366–1378. http://doi.org/10.1152/jn.01009.2011

Marioni, R. E., Proust-Lima, C., Amieva, H., Brayne, C., Matthews, F. E., Dartigues, J.-F., & Jacqmin-Gadda, H. (2015). Social activity, cognitive decline and dementia risk: a 20-year prospective cohort study. *BMC Public Health*, *15*(1), 1089. http://doi.org/10.1186/s12889-015-2426-6

Masliah, E., Honer, W. G., Mallory, M., Voigt, M., Kushner, P., Hansen, L., & Terry, R. (1994). Topographical distribution of synaptic-associated proteins in the neuritic plaques of Alzheimer's disease hippocampus. *Acta Neuropathologica*,

87(2), 135–142. http://doi.org/10.1007/BF00296182

Mattson, M. P., Maudsley, S., & Martin, B. (2004). BDNF and 5-HT: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends in Neurosciences*. http://doi.org/10.1016/j.tins.2004.08.001

McAllister, a K., Lo, D. C., & Katz, L. C. (1995). Neurotrophins regulate dendritic growth in developing visual cortex. *Neuron*, *15*(4), 791–803. http://doi.org/0896-6273(95)90171-X [pii]

McGeer, P. L., Itagaki, S., Tago, H., & McGeer, E. G. (1987). Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. *Neuroscience Letters*, *79*(1–2), 195–200. http://doi.org/10.1016/0304-3940(87)90696-3

McGowan, E., Eriksen, J., & Hutton, M. (2006). A decade of modeling Alzheimer's disease in transgenic mice. *Trends in Genetics*. http://doi.org/10.1016/j.tig.2006.03.007

McGuire, L. C., Ford, E. S., & Ajani, U. a. (2006). Cognitive functioning as a predictor of functional disability in later life. *The American Journal of Geriatric Psychiatry : Official Journal of the American Association for Geriatric Psychiatry*, *14*(1), 36–42. http://doi.org/10.1097/01.JGP.0000192502.10692.d6

Meda, L., Baron, P., Prat, E., Scarpini, E., Scarlato, G., Cassatella, M. A., & Rossi, F. (1999). Proinflammatory profile of cytokine production by human monocytes and murine microglia stimulated with β-amyloid[25-35]. *Journal of Neuroimmunology*, *93*(1–2), 45–52. http://doi.org/10.1016/S0165-5728(98)00188-X

Merino-Serrais, P., Knafo, S., Alonso-Nanclares, L., Fernaud-Espinosa, I., & Defelipe, J. (2011). Layer-specific alterations to CA1 dendritic spines in a mouse model of Alzheimer's disease. *Hippocampus*, *21*(10), 1037–1044.

http://doi.org/10.1002/hipo.20861

Meyer-Luehmann, M., Spires-Jones, T. L., Prada, C., Garcia-Alloza, M., de Calignon, A., Rozkalne, A., … Hyman, B. T. (2008). Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature*, *451*(7179), 720–724. http://doi.org/10.1038/nature06616

Mirochnic, S., Wolf, S., Staufenbiel, M., & Kempermann, G. (2009). Age effects on the regulation of adult hippocampal neurogenesis by physical activity and environmental enrichment in the APP23 mouse model of Alzheimer disease. *Hippocampus*, *19*(10), 1008–1018. http://doi.org/10.1002/hipo.20560

Mitew, S., Kirkcaldie, M. T., Dickson, T. C., & Vickers, J. C. (2013). Neurites containing the neurofilament-triplet proteins are selectively vulnerable to cytoskeletal pathology in Alzheimer's disease and transgenic mouse models. *Front Neuroanat*, *7*, 30. http://doi.org/10.3389/fnana.2013.00030

Mitew, S., Kirkcaldie, M. T. K., Dickson, T. C., & Vickers, J. C. (2013). Altered synapses and gliotransmission in alzheimer's disease and AD model mice. *Neurobiology of Aging*, *34*(10), 2341–2351. http://doi.org/10.1016/j.neurobiolaging.2013.04.010

Morris, G. P., Clark, I. A., Zinn, R., & Vissel, B. (2013). Microglia: A new frontier for synaptic plasticity, learning and memory, and neurodegenerative disease research. *Neurobiology of Learning and Memory*, *105*, 40–53. http://doi.org/10.1016/j.nlm.2013.07.002

Morris, J. C., Storandt, M., Miller, J. P., McKeel, D. W., Price, J. L., Rubin, E. H., & Berg, L. (2001). Mild cognitive impairment represents early-stage Alzheimer disease. *Archives of Neurology*, *58*(3), 397–405. http://doi.org/10.1001/archneur.58.3.397

Morrison, J. H., & Baxter, M. G. (2014). Synaptic Health. *JAMA Psychiatry*, *71*(7), 7–9. http://doi.org/10.1001/jamapsychiatry.2014.380

Moser, M. B., Trommald, M., & Andersen, P. (1994). An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(26), 12673–5. http://doi.org/10.1073/pnas.91.26.12673

Moser, M. B., Trommald, M., Egeland, T., & Andersen, P. (1997). Spatial training in a complex environment and isolation alter the spine distribution differently in rat CA1 pyramidal cells. *Journal of Comparative Neurology*, *380*(3), 373–381. http://doi.org/10.1002/(SICI)1096-9861(19970414)380:3<373::AID-CNE6>3.0.CO;2-#

Mosher, K. I., & Wyss-Coray, T. (2014). Microglial dysfunction in brain aging and Alzheimer's disease. *Biochemical Pharmacology, 88*(4), 594-604. http://doi.org/10.1016/j.bcp.2014.01.008

Murer, M. G., Yan, Q., & Raisman-Vozari, R. (2001). Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Progress in Neurobiology, 63*(1), 71-124. http://doi.org/10.1016/S0301-0082(00)00014-9

Nelson, D. H. (1972). Regulation of glucocorticoid release. *The American Journal of Medicine*, *53*(5), 590–594. http://doi.org/10.1016/0002-9343(72)90155-6

Nelson, P. T., Braak, H., & Markesbery, W. R. (2009). Neuropathology and Cognitive Impairment in Alzheimer Disease: A Complex but Coherent Relationship. *Journal of Neuropathology & Experimental Neurology*, *68*(1), 1–14. http://doi.org/10.1097/NEN.0b013e3181919a48

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Neuroforum*, *11*(3), 95–96. http://doi.org/10.1126/science.1110647

Nishitomi, K., Sakaguchi, G., Horikoshi, Y., Gray, A. J., Maeda, M., Hirata-Fukae, C., … Matsuoka, Y. (2006). BACE1 inhibition reduces endogenous Abeta and alters APP processing in wild-type mice2. *Journal of Neurochemistry*, *99*(6), 1555–1563. http://doi.org/10.1111/j.1471-4159.2006.04178.x

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews. Neuroscience*, *7*(September), 697–709. http://doi.org/10.1038/nrn1970

Nithianantharajah, J., Levis, H., & Murphy, M. (2004). Environmental enrichment results in cortical and subcortical changes in levels of synaptophysin and PSD-95 proteins. *Neurobiology of Learning and Memory*, *81*(3), 200–210. http://doi.org/10.1016/j.nlm.2004.02.002

Nitz, D., & McNaughton, B. (2004). Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. *Journal of Neurophysiology*, *91*(2), 863–872. http://doi.org/10.1152/jn.00614.2003

Novkovic, T., Mittmann, T., & Manahan-Vaughan, D. (2015). BDNF contributes to the facilitation of hippocampal synaptic plasticity and learning enabled by environmental enrichment. *Hippocampus*, *25*(1), 1–15. http://doi.org/10.1002/hipo.22342

O'Brien, R. J., & Wong, P. C. (2011). Amyloid Precursor Protein Processing and Alzheimer's Disease. *Annual Review of Neuroscience*, (March), 183–202. http://doi.org/10.1146/annurev-neuro-061010-113613

Palop, J. J., & Mucke, L. (2010). Amyloid-[beta]-induced neuronal dysfunction in

Alzheimer's disease: from synapses toward neural networks. *Nature Neuroscience*, *13*(7), 812–818. http://doi.org/10.1038/nn.2583

Pang, T. Y. C., & Hannan, A. J. (2013). Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. *Neuropharmacology*. http://doi.org/10.1016/j.neuropharm.2012.06.029

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., … Gross, C. T. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science (New York, N.Y.)*, *333*(6048), 1456–8. http://doi.org/10.1126/science.1202529

Park, H., & Poo, M. M. (2013). Neurotrophin regulation of neural circuit development and function. *Nat Rev Neurosci*, *14*(1), 7–23. http://doi.org/10.1038/nrn3379

Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R., Lafaille, J. J., … Gan, W. B. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell*, *155*(7), 1596–1609. http://doi.org/10.1016/j.cell.2013.11.030

Patrylo, P. R., & Williamson, A. (2007). The effects of aging on dentate circuitry and function. *Progress in Brain Research, 163,* 679-696. http://doi.org/10.1016/S0079-6123(07)63037-4

Patterson, S. L. (2015). Immune dysregulation and cognitive vulnerability in the aging brain: Interactions of microglia, IL-1β, BDNF and synaptic plasticity. *Neuropharmacology*, (Part A), 11–18. http://doi.org/10.1016/j.neuropharm.2014.12.020

Paxinos, G., & Franklin, K. (2008). *The Mouse Brain in Stereotaxic Coordinates, Compact | 978-0-12-374244-5 | Elsevier*. *The Mouse Brain in Stereotaxic Coordinates*. Retrieved from https://www.elsevier.com/books/the-mouse-brain-in-

stereotaxic-coordinates-compact/paxinos/978-0-12-374244-5

Perlmutter, L. S., Barron, E., & Chui, H. C. (1990). Morphologic association between microglia and senile plaque amyloid in Alzheimer's disease. *Neuroscience Letters*, *119*(1), 32–36. http://doi.org/10.1016/0304-3940(90)90748-X

Perry, V. H., Cunningham, C., & Holmes, C. (2007). Systemic infections and inflammation affect chronic neurodegeneration. *Nature Reviews. Immunology*, *7*(2), 161–167. http://doi.org/10.1038/nri2015

Perry, V. H., Hume, D. A., & Gordon, S. (1985). Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience*, *15*(2), 313–326. http://doi.org/10.1016/0306-4522(85)90215-5

Price, J. L., & Morris, J. C. (1999). Tangles and plaques in nondemented aging and "preclinical" alzheimer's disease. *Annals of Neurology*, *45*(3), 358–368. http://doi.org/10.1002/1531-8249(199903)45:3<358::AID-ANA12>3.0.CO;2-X

Prince, M., Wimo, A., Guerchet, M., Gemma-Claire, A., Wu, Y.-T., & Prina, M. (2015). World Alzheimer Report 2015: The Global Impact of Dementia - An analysis of prevalence, incidence, cost and trends. *Alzheimer's Disease International*, 84. http://doi.org/10.1111/j.0963-7214.2004.00293.x

Prokop, S., Miller, K. R., & Heppner, F. L. (2013). Microglia actions in Alzheimer's disease. *Acta Neuropathologica*. http://doi.org/10.1007/s00401-013-1182-x

Puzzo, D., Gulisano, W., Palmeri, A., & Arancio, O. (2015). Rodent models for Alzheimer's disease drug discovery. *Expert Opinion on Drug Discovery*, *10*(7), 703–11. http://doi.org/10.1517/17460441.2015.1041913

Ramírez-Rodríguez, G., Ocaña-Fernández, M. A., Vega-Rivera, N. M., Torres-Pérez, O. M., Gómez-Sánchez, A., Estrada-Camarena, E., & Ortiz-López, L. (2014). Environmental enrichment induces neuroplastic changes in middle age female

BalbC mice and increases the hippocampal levels of BDNF, p-Akt and p-MAPK1/2. *Neuroscience*, *260*, 158–170. http://doi.org/10.1016/j.neuroscience.2013.12.026

Rampon, C., Tang, Y. P., Goodhouse, J., Shimizu, E., Kyin, M., & Tsien, J. Z. (2000). Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nature Neuroscience*, *3*(3), 238–244. http://doi.org/10.1038/72945

Ransohoff, R. M., & Perry, V. H. (2009). Microglial Physiology: Unique Stimuli, Specialized Responses. *Annual Review of Immunology*, *27*(1), 119–145. http://doi.org/10.1146/annurev.immunol.021908.132528

Rasmuson, S., Andrew, R., Näsman, B., Seckl, J. R., Walker, B. R., & Olsson, T. (2001). Increased glucocorticoid production and altered cortisol metabolism in women with mild to moderate Alzheimer's disease. *Biological Psychiatry*, *49*(6), 547–552. http://doi.org/10.1016/S0006-3223(00)01015-5

Rehm, H., Wiedenmann, B., & Betz, H. (1986). Molecular characterization of synaptophysin, a major calcium-binding protein of the synaptic vesicle membrane. *The EMBO Journal*, *5*(3), 535–41. http://doi.org/10.1128/IAI.69.3.1244

Rodríguez, J. J., Noristani, H. N., & Verkhratsky, A. (2015). Microglial response to Alzheimer's disease is differentially modulated by voluntary wheel running and enriched environments. *Brain Structure & Function*, *220*(2), 941–953. http://doi.org/10.1007/s00429-013-0693-5

Rohe, M., Synowitz, M., Glass, R., Paul, S. M., Nykjaer, A., & Willnow, T. E. (2009). Brain-derived neurotrophic factor reduces amyloidogenic processing through control of SORLA gene expression. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(49), 15472–8.

http://doi.org/10.1523/JNEUROSCI.3960-09.2009

Roozendaal, B., Phillips, R. G., Power, a E., Brooke, S. M., Sapolsky, R. M., & McGaugh, J. L. (2001). Memory retrieval impairment induced by hippocampal CA3 lesions is blocked by adrenocortical suppression. *Nature Neuroscience*, *4*, 1169–1171. http://doi.org/10.1038/nn766

Rosenzweig, M. R. (1996). Aspects of the search for neural mechanisms of memory. *Annual Review of Psychology*, *47*, 1–32. http://doi.org/10.1146/annurev.psych.47.1.1

Rothman, S. M., Herdener, N., Camandola, S., Texel, S. J., Mughal, M. R., Cong, W. N., … Mattson, M. P. (2012). 3xTgAD mice exhibit altered behavior and elevated Aβ after chronic mild social stress. *Neurobiology of Aging*, *33*(4). http://doi.org/10.1016/j.neurobiolaging.2011.07.005

Rubio-Perez, J. M., & Morillas-Ruiz, J. M. (2012). A Review: Inflammatory Process in Alzheimer's Disease, Role of Cytokines. *The Scientific World Journal*, *2012*, 1–15. http://doi.org/10.1100/2012/756357

Sale, A., Berardi, N., & Maffei, L. (2009). Enrich the environment to empower the brain. *Trends in Neurosciences*. http://doi.org/10.1016/j.tins.2008.12.004

Salthouse, T. A. (2011). Neuroanatomical substrates of age-related cognitive decline. *Psychological Bulletin*, *137*(5), 753–784. http://doi.org/10.1037/a0023262.Neuroanatomical

Satz, P., Benson, D. F., Crinella, F., D 'elia, L., Earnest, K., Evans, G., … Jolyn, L. (1993). Brain Reserve Capacity on Symptom Onset After Brain Injury: A Formulation and Review of Evidence for Threshold Theory. *Neuropsychology*, *7*(3), 273–295. http://doi.org/10.1037/0894-4105.7.3.273

Scarmeas, N., Levy, G., Tang, M. X., Manly, J., & Stern, Y. (2001). Influence of leisure

activity on the incidence of Alzheimer's disease. *Neurology*, *57*(12), 2236–42. http://doi.org/10.1212/WNL.57.12.2236

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., … Stevens, B. (2012). Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron*, *74*(4), 691–705. http://doi.org/10.1016/j.neuron.2012.03.026

Scheff, S. W., & Price, D. a. (2006). Alzheimer's disease-related alterations in synaptic density: neocortex and hippocampus. *Journal of Alzheimer's Disease : JAD*, *9*, 101–115. http://doi.org/Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review

Scheff, S. W., Price, D. a., Schmitt, F. a., Dekosky, S. T., & Mufson, E. J. (2007). Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology*, *68*(18), 1501–1508. http://doi.org/10.1212/01.wnl.0000260698.46517.8f

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., … Younkin, S. (1996). Secreted amyloid [beta]-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med*, *2*(8), 864–870. Retrieved from http://dx.doi.org/10.1038/nm0896-864

Segovia, G., del Arco, A., & Mora, F. (2009). Environmental enrichment, prefrontal cortex, stress, and aging of the brain. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *116*(8), 1007–16. http://doi.org/10.1007/s00702-009-0214-0

Selkoe, D. J. (2002). Alzheimer's disease is a synaptic failure. *Science (New York, N.Y.)*, *298*(5594), 789–791. http://doi.org/10.1126/science.1074069

Serrano-Pozo, A., Gómez-Isla, T., Growdon, J. H., Frosch, M. P., & Hyman, B. T.

(2013). A phenotypic change but not proliferation underlies glial responses in Alzheimer disease. *American Journal of Pathology*, *182*(6), 2332–2344. http://doi.org/10.1016/j.ajpath.2013.02.031

Sharp, E. S., & Gatz, M. (2011). Relationship between education and dementia: an updated systematic review. *Alzheimer Disease and Associated Disorders*, *25*(4), 289–304. http://doi.org/10.1097/WAD.0b013e318211c83c

Sheng, J. G., Mrak, R. E., & Griffin, W. S. T. (1998). Enlarged and phagocytic, but not primed, interleukin-1 alpha-immunoreactive microglia increase with age in normal human brain. *Acta Neuropathologica*, *95*(3), 229–234. http://doi.org/10.1007/s004010050792

Sherrington, R., Froelich, S., Sorbi, S., Campion, D., Chi, H., Rogaeva, E. A., … St George-Hyslop, P. H. (1996). Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. *Human Molecular Genetics*, *5*(7), 985–988. http://doi.org/10.1093/hmg/5.7.985

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., … St George-Hyslop, P. H. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*. http://doi.org/10.1038/375754a0

Sierra, A., Gottfried-Blackmore, A. C., Mcewen, B. S., & Bulloch, K. (2007). Microglia derived from aging mice exhibit an altered inflammatory profile. *GLIA*, *55*(4), 412–424. http://doi.org/10.1002/glia.20468

Silverman, M. N., & Sternberg, E. M. (2012). Glucocorticoid regulation of inflammation and its functional correlates: From HPA axis to glucocorticoid receptor dysfunction. *Annals of the New York Academy of Sciences*. http://doi.org/10.1111/j.1749-6632.2012.06633.x

Sommer, C., Straehle, C., Kothe, U., & Hamprecht, F. A. (2011). Ilastik: Interactive

learning and segmentation toolkit. In *Proceedings - International Symposium on Biomedical Imaging* (pp. 230–233). http://doi.org/10.1109/ISBI.2011.5872394

Sorrentino, P., Iuliano, A., Polverino, A., Jacini, F., & Sorrentino, G. (2014). The dark sides of amyloid in Alzheimer's disease pathogenesis. *FEBS Letters*. http://doi.org/10.1016/j.febslet.2013.12.038

Sosa-Ortiz, A. L., Acosta-Castillo, I., & Prince, M. J. (2012). Epidemiology of Dementias and Alzheimer's Disease. *Archives of Medical Research*. http://doi.org/10.1016/j.arcmed.2012.11.003

Southam, K. A., Vincent, A. J., & Small, D. H. (2016). Do Microglia Default on Network Maintenance in Alzheimer's Disease? *Journal of Alzheimer's Disease*, *51*(3), 657–669. http://doi.org/10.3233/JAD-151075

Stern, Y. (2002). What is cognitive reserve? Theory and research application of the reserve concept. *Journal of the International Neuropsychological Society : JINS*, *8*(3), 448–460. http://doi.org/10.1017/S1355617702813248

Stern, Y. (2012). Cognitive reserve in ageing and Alzheimer's disease. *The Lancet Neurology*. http://doi.org/10.1016/S1474-4422(12)70191-6

Stevens, B., Allen, N. J., Vazquez, L. E., Howell, G. R., Christopherson, K. S., Nouri, N., … Barres, B. A. (2007). The Classical Complement Cascade Mediates CNS Synapse Elimination. *Cell*, *131*(6), 1164–1178. http://doi.org/10.1016/j.cell.2007.10.036

Streit, W. J. (2006). Microglial senescence: does the brain's immune system have an expiration date? *Trends in Neurosciences*, *29*(9), 506–510. http://doi.org/10.1016/j.tins.2006.07.001

Streit, W. J., Mrak, R. E., & Griffin, W. S. T. (2004). Microglia and neuroinflammation: a pathological perspective. *Journal of Neuroinflammation*,

1(doi:10.1186/1742-2094-1-14), 4 pages. http://doi.org/10.1186/1742-2094-1-14

Streit, W. J., Xue, Q.-S., Tischer, J., & Bechmann, I. (2014). Microglial pathology. *Acta Neuropathologica Communications*, *2*, 142. http://doi.org/10.1186/s40478-014-0142-6

Summers, MJ; Saunders, NL; Valenzuela, MJ; Summers, JJ; Ritchie, K; Robinson, A; Vickers, J. (2013). The Tasmanian Healthy Brain Project (THBP): A prospective longitudinal examination of the effect of university-level education in older adults in preventing age-related cognitive decline and reducing the risk of dementia. *International Psychogeriatrics*, *25*(7), 1145–1155.

Sze, C. I., Troncoso, J. C., Kawas, C., Mouton, P., Price, D. L., & Martin, L. J. (1997). Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *Journal of Neuropathology and Experimental Neurology*, *56*(8), 933–44. http://doi.org/10.1097/00005072-199708000-00011

Terry, R. D., Masliah, E., Salmon, D. P., Butters, N., DeTeresa, R., Hill, R., … Katzman, R. (1991). Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Annals of Neurology*, *30*(4), 572–580. http://doi.org/10.1002/ana.410300410

Thal, D. R., Walter, J., Saido, T. C., & Fändrich, M. (2015). Neuropathology and biochemistry of Aβ and its aggregates in Alzheimer's disease. *Acta Neuropathologica*. http://doi.org/10.1007/s00401-014-1375-y

Thornton, E., Vink, R., Blumbergs, P. C., & Van Den Heuvel, C. (2006). Soluble amyloid precursor protein alpha reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Research*, *1094*(1), 38–46. http://doi.org/10.1016/j.brainres.2006.03.107

Tremblay, M.-È., Lecours, C., Samson, L., Sánchez-Zafra, V., & Sierra, A. (2015). From the Cajal alumni Achúcarro and Río-Hortega to the rediscovery of never-resting microglia. *Frontiers in Neuroanatomy*, *9*, 45. http://doi.org/10.3389/fnana.2015.00045

Tremblay, M. Ě., Lowery, R. L., & Majewska, A. K. (2010). Microglial interactions with synapses are modulated by visual experience. *PLoS Biology*, *8*(11). http://doi.org/10.1371/journal.pbio.1000527

Turner, A. M., & Greenough, W. T. (1985). Differential rearing effects on rat visual cortex synapses. I. Synaptic and neuronal density and synapses per neuron. *Brain Research*, *329*(1–2), 195–203. http://doi.org/10.1016/0006-8993(85)90525-6

Uylings, H. B. M., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex? *Behavioural Brain Research*. http://doi.org/10.1016/j.bbr.2003.09.028

Valenzuela, M., Brayne, C., Sachdev, P., Wilcock, G., & Matthews, F. (2011). Cognitive lifestyle and long-term risk of dementia and survival after diagnosis in a multicenter population-based cohort. *American Journal of Epidemiology*, *173*(9), 1004–12. http://doi.org/10.1093/aje/kwq476

Valenzuela, M. J., & Sachdev, P. (2006). Brain reserve and dementia: a systematic review. *Psychological Medicine*, *36*(4), 441–54. http://doi.org/10.1017/S0033291705006264

Van De Werd, H. J. J. M., Rajkowska, G., Evers, P., & Uylings, H. B. M. (2010). Cytoarchitectonic and chemoarchitectonic characterization of the prefrontal cortical areas in the mouse. *Brain Structure and Function*, *214*(4), 339–353. http://doi.org/10.1007/s00429-010-0247-z

van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews. Neuroscience*, *1*(December), 191–198.

http://doi.org/10.1038/35044558

Verghese, P. B., Castellano, J. M., & Holtzman, D. M. (2011). Apolipoprotein E in Alzheimer's disease and other neurological disorders. *The Lancet Neurology*. http://doi.org/10.1016/S1474-4422(10)70325-2

Verret, L., Krezymon, A., Halley, H., Trouche, S., Zerwas, M., Lazouret, M., … Rampon, C. (2013). Transient enriched housing before amyloidosis onset sustains cognitive improvement in Tg2576 mice. *Neurobiology of Aging*, *34*(1), 211–225. http://doi.org/10.1016/j.neurobiolaging.2012.05.013

Vickers, J. C., Dickson, T. C., Adlard, P. a, Saunders, H. L., King, C. E., & McCormack, G. (2000). The cause of neuronal degeneration in Alzheimer's disease [Review]. *Progress in Neurobiology*, *60*(2), 139–165. http://doi.org/10.1016/S0301-0082(99)00023-4

Villemagne, V. L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K. A., Salvado, O., … Masters, C. L. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurology*, *12*(4), 357–67. http://doi.org/10.1016/S1474-4422(13)70044-9

von Bernhardi, R. (2007). Glial cell dysregulation: A new perspective on Alzheimer disease. *Neurotoxicity Research*, *12*(4), 215–232. http://doi.org/10.1007/BF03033906

Vukovic, J., Colditz, M. J., Blackmore, D. G., Ruitenberg, M. J., & Bartlett, P. F. (2012). Microglia Modulate Hippocampal Neural Precursor Activity in Response to Exercise and Aging. *Journal of Neuroscience*, *32*(19), 6435–6443. http://doi.org/10.1523/JNEUROSCI.5925-11.2012

Wake, H., Moorhouse, A. J., Jinno, S., Kohsaka, S., & Nabekura, J. (2009). Resting microglia directly monitor the functional state of synapses in vivo and determine

the fate of ischemic terminals. *J Neurosci*, *29*(13), 3974–3980. http://doi.org/10.1523/JNEUROSCI.4363-08.2009

Wang, H.-X., Jin, Y., Hendrie, H. C., Liang, C., Yang, L., Cheng, Y., … Gao, S. (2013). Late life leisure activities and risk of cognitive decline. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *68*(2), 205–13. http://doi.org/10.1093/gerona/gls153

Wang, H., Megill, A., He, K., Kirkwood, A., & Lee, H. K. (2012). Consequences of inhibiting amyloid precursor protein processing enzymes on synaptic function and plasticity. *Neural Plasticity*. http://doi.org/10.1155/2012/272374

Wang, J. H., Ma, Y. Y., & van den Buuse, M. (2006). Improved spatial recognition memory in mice lacking adenosine A2A receptors. *Experimental Neurology*, *199*(2), 438–45. http://doi.org/10.1016/j.expneurol.2006.01.005

Wang, J., Tanila, H., Puoliväli, J., Kadish, I., & Van Groen, T. (2003). Gender differences in the amount and deposition of amyloidβ in APPswe and PS1 double transgenic mice. *Neurobiology of Disease*, *14*(3), 318–327. http://doi.org/10.1016/j.nbd.2003.08.009

Weintraub, S., Wicklund, A. H., & Salmon, D. P. (2012). The neuropsychological profile of Alzheimer disease. *Cold Spring Harbor Perspectives in Medicine*. http://doi.org/10.1101/cshperspect.a006171

West, M. J., Bach, G., Søderman, A., & Jensen, J. L. (2009). Synaptic contact number and size in stratum radiatum CA1 of APP/PS1ΔE9 transgenic mice. *Neurobiology of Aging*, *30*(11), 1756–1776. http://doi.org/10.1016/j.neurobiolaging.2008.01.009

Williamson, L. L., Chao, A., & Bilbo, S. D. (2012). Environmental enrichment alters glial antigen expression and neuroimmune function in the adult rat hippocampus. *Brain, Behavior, and Immunity*, *26*(3), 500–510.

http://doi.org/10.1016/j.bbi.2012.01.003

Williamson, L. L., Sholar, P. W., Mistry, R. S., Smith, S. H., & Bilbo, S. D. (2011). Microglia and memory: modulation by early-life infection. *J Neurosci*, *31*(43), 15511–15521. http://doi.org/10.1523/JNEUROSCI.3688-11.2011

Wolf, S. a., Kronenberg, G., Lehmann, K., Blankenship, A., Overall, R., Staufenbiel, M., & Kempermann, G. (2006). Cognitive and Physical Activity Differently Modulate Disease Progression in the Amyloid Precursor Protein (APP)-23 Model of Alzheimer's Disease. *Biological Psychiatry*, *60*(Mdc), 1314–1323. http://doi.org/10.1016/j.biopsych.2006.04.004

Woodhouse, A., Vickers, J. C., Adlard, P. A., & Dickson, T. C. (2009). Dystrophic neurites in TgCRND8 and Tg2576 mice mimic human pathological brain aging. *Neurobiology of Aging*, *30*(6), 864–874. http://doi.org/10.1016/j.neurobiolaging.2007.09.003

Xu, H., Gelyana, E., Rajsombath, M., Yang, T., Li, S., & Selkoe, D. (2016). Environmental Enrichment Potently Prevents Microglia-Mediated Neuroinflammation by Human Amyloid β-Protein Oligomers. *The Journal of Neuroscience*, *36*(35), 9041–9056. http://doi.org/10.1523/JNEUROSCI.1023-16.2016

Xu, W., Yu, J.-T., Tan, M.-S., & Tan, L. (2015). Cognitive reserve and Alzheimer's disease. *Molecular Neurobiology*, *51*(1), 187–208. http://doi.org/10.1007/s12035-014-8720-y

Yoshii, A., & Constantine-Paton, M. (2010). Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Developmental Neurobiology*. http://doi.org/10.1002/dneu.20765

Youmans, K. L., Tai, L. M., Kanekiyo, T., Stine, W. B., Michon, S.-C., Nwabuisi-

Heath, E., … Ladu, M. J. (2012). Intraneuronal Abeta detection in 5xFAD mice by a new Abeta-specific antibody. *Molecular Neurodegeneration*, *7*(1), 8. http://doi.org/10.1186/1750-1326-7-8

Zhang, L., Xing, Y., Ye, C. F., Ai, H. X., Wei, H. F., & Li, L. (2006). Learning-memory deficit with aging in APP transgenic mice of Alzheimer's disease and intervention by using tetrahydroxystilbene glucoside. *Behavioural Brain Research*, *173*(2), 246–254. http://doi.org/10.1016/j.bbr.2006.06.034

Ziv, Y., Ron, N., Butovsky, O., Landa, G., Sudai, E., Greenberg, N., … Schwartz, M. (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci*, *9*(2), 268–275. http://doi.org/10.1038/nn1629

Appendix A

Stuart, King, Fernandez-Martos, Dittmann, Summers, & Vickers (2016), reprint of accepted article